SPECIATION OF PHOSPHORUS IN MANURE- AND INORGANIC FERTILIZER-AMENDED SASKATCHEWAN SOILS

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ABSTRACT

Concern over excess phosphorus (P) input and loading in some soils of the Canadian prairie region has led to a need for a better understanding of the fate of added manure and fertilizer P. Information on the effects of manure application over long term (i.e., years) and short term (i.e., weeks, months) as related to management practices and manure form is still lacking. Knowledge of the P forms and species present in soil following application of manure and inorganic P fertilizers, and linking this to potential P availability and mobility is needed to make sound P management recommendations. The objective of this thesis project was to assess the speciation of soil P in different manures and inorganic fertilizer-amended Saskatchewan soils as affected by time, presence and absence of plants, landscape position, soil type, and management practices including rate and placement. Three studies were conducted (growth chamber and field-based experiments) to study P behavior in soils. These studies closely followed a time scale, beginning with speciation and fate followed over the very short-term (i.e., days to weeks) to a short-term period (i.e., months to a year) following amendment application, and finally the effects of repeated annual manure additions made over the long term (i.e., 11 years). Soils used in this study were loamy textured Brown and Black Chernozems. Solid cattle manure (SCM) and liquid hog manure (LHM) were applied at low and high rates in the very short-term and long-term studies. The low rate of SCM and LHM application was 7.6 T ha^{-1} yr^{-1} (dry weight) and 37,000 L ha^{-1} yr^{-1}, respectively which was equivalent to approximately 100 kg total N ha^{-1} yr^{-1} application (agronomic N rate). The high rate was four times this amount. Inorganic fertilizer (mono-ammonium phosphate blended with urea) at rate of 54 kg N ha^{-1} and 12 kg P ha^{-1} and SCM at rate of 60 T ha^{-1} were applied in the short-term study.

A sequential chemical extraction procedure was used to fractionate P in very short-term and short-term studies and different soil test phosphorus (STP) methods were used to determine effects on the labile P in the long-term study. Changes in P speciation with time and their relative proportions in fertilizered soils were also assessed using the synchrotron-based X-ray absorption near-edge structure (XANES) spectroscopy in all studies.

This study has revealed that manure, especially SCM elevates labile P over all time frames examined. Over the very short-term (i.e., weeks to a month), P was added as manure tends to remain in labile forms like brushite and adsorbed P that is accessible to plants for uptake. In the manure band (months to a year), manure P was relatively unchanged over a period of months while in the mono-ammonium phosphate (MAP) fertilizer band, adsorbed and Ca-P was readily formed from fertilizer P. Aging over several years (one to eleven years), along with high soil pH and high Ca:P ratio enhanced formation of more stable Ca-P minerals like apatite, especially in SCM amended soil. Overall, the unique combined use of wet chemical analysis and synchrotron-based techniques in this thesis research has improved our understanding of fate and transformation of P added to prairie soils. It is suggested that future studies of fate of applied P in soil also utilize a combination of wet chemical and spectroscopic techniques, as this was shown to be a rewarding approach.

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This entire process would not have been possible without the support of my wife, parents, brothers, and uncle. The inspiration and support from them was always unconditional and quantified. I would like to thank all of you for your invaluable support and suggestions along the way.

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Thanks to all of them who were there for me at different times and their names are not mentioned here. Lastly, I am grateful that God has placed me here and cared for me.

DEDICATION

I dedicate this Dissertation to my loving Wife, Daughter, Parents-in-law, Mothers, Brothers, and Uncle

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LIST OF ABBREVIATIONS

CLS	Canadian light source
CEC	Cation exchange capacity
DCP	Dicalcium phosphate
DCPD	Dicalcium phosphate dihydrate
EC	Electrical conductivity
EXAFS	Extended x-ray absorption fine structure
FY	Fluorescence yield
HAP	Hydroxyapatite
HCl	Hydrochloric acid
HFO	Phosphate adsorbed on an Fe oxide
KM	Kelowna method
LC	Linear combination
MAP	Mono-ammonium phosphate
LHM	Liquid hog manure
NMR	Nuclear magnetic resonance spectroscopy
NaOH	Sodium hydroxide
OC	Organic carbon
OM	Organic matter
P	Significance level
PAMI	Prairie agricultural machinery institute
PFY	Partial fluorescence yield
P_i	Inorganic phosphorus
P_o	Organic P
P_t	Total P
PRS	Plant root simulator
SCM	Solid cattle manure
SD	Standard deviation
SEM	Standard error of mean
STP	Soil test phosphorus
SXRMB	Soft X-ray micro-characterization beamline
TC	Total carbon
TEY	Total electron yield
XANES	X-ray absorption near edge structure
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction

CHAPTER 1.0

INTRODUCTION

Meeting increasing food demand of the growing global population while maintaining and improving environmental quality is a significant challenge of agronomy. It is estimated that crop production needs to be increased by about 30 to 40% more on the same agricultural land base in order to meet the increasing demand for food (Havlin et al., 2005). Organic and inorganic fertilizers are used to meet plant nutrient requirements and thus enable increased crop production all over the world. Organic sources of nutrients have been applied on agricultural land for many years as a valuable source of plant nutrients (Gburek et al., 2000; Havlin et al., 2005). Animal manure is one of the most widely used organic sources of plant nutrients. It is one of the main land-applied organic sources of plant phosphorus (P) for crop production used globally and for Canadian prairie regions. The gradual increase in intensive livestock production in the prairie regions has promoted the increased use of animal manures as a P source for crop production (Stumborg, 2008).

Unfortunately, the nutrients in manure are not balanced for optimal crop growth as they are in commercial inorganic fertilizers. Commercialized inorganic products can be blended in various proportions to achieve the desired nutrient balance required by plants. For example, cattle feedlot manure contains about four times more nitrogen (N) than that of P while plants require about eight times more N than P (Qian et al., 2004). Therefore, when manure is applied based on plant requirement for one nutrient, other nutrients are usually either over- or under-applied. Nitrogen-based application rates are used in Saskatchewan (Saskatchewan Ministry of Agriculture, 2006) and Alberta. Thus, the continuous application of manure based on crop N requirement results in the accumulation of P in soil (Simard et al., 1995; Whalen and Chang,

2001). The elevated soil concentration increases the risk of P loss from the soil system through erosion, runoff, and leaching processes. High amounts of labile (available) P in soil aggravate the risk (Qian et al., 2004).

Phosphorus is present in manure in both inorganic and organic forms (Sharpley and Moyer, 2000; Eghball, 2003). Many researchers (Gerritse and Vriesema, 1984; Barnett, 1994; Leinweber, 1996) have reported that about 50% of manure P is available for crop use in the year of application and inorganic forms are very sensitive to runoff loss after land application (Dou et al., 2000). Continued application of manure increases both total and soil test P concentration in soil (Sharpley et al., 1984) and also decreases the capacity of soil to hold the P in less soluble, stable forms (Kashem et al., 2004). Generally, the higher the level of soil test P (STP), the higher the risk of P losses from the soil (Sibbesen and Sharpley, 1997; Sims, 1998; Kashem et al., 2004; Hao et al., 2008) which may pose risks to the health of aquatic ecosystems (Ajiboye, 2008).

A great deal of work has been conducted to assess the nutrient value of manure (Evans et al., 1977; Baldwin, 1983; Safley et al., 1986; Beauchamp, 1987) under different management practices (Sutton et al., 1982; Sawyer et al., 1990; Stumborg and Schoenau, 2008). However, studies on the effects of animal manure application on P speciation in calcareous prairie soils are few (Michael et al., 2000; Qian et al., 2004). Information on P speciation after long-term (more than 10 years) repeated manure applications alongside short-term effects of a single application followed over weeks or months is needed to fully understand the fate of the P applied as animal manure in soils of the prairies.

There are many widely used STP methods, along with sequential chemical extractions that have been used to measure and fractionate soil P (Kashem et al., 2004; Hao et al., 2008),

which may be valuable in better understanding the fate of P in prairie soils. While soil test P and sequential chemical extraction methods can operationally separate P into different inorganic and organic P fractions, they cannot conclusively identify the specific P compounds present in soil (Zamuner et al., 2008). The availability and transformations of applied P that occur in soil mainly depends on their chemical form, so accurate characterization of soil P forms is essential in understanding the cycling of soil P. Characterization of different forms of P and understanding their chemistry is essential to understanding and predicting the fate and transformation of the applied inorganic or organic fertilizer P.

The chemical speciation and identification of individual P compounds present in soils may be further advanced by using molecular-scale spectroscopic techniques including X-ray Absorption Spectroscopy (XAS) (Fendorf et al., 1994; Hesterberg et al., 1999; Beauchemin et al., 2003; Shober et al., 2006), differential X-ray Diffractometry (XRD) (Ippolito et al., 2003; Huang and Shenker, 2004), and Nuclear Magnetic Resonance Spectroscopy (NMR) (Hinedi et al., 1989; Frossard et al., 1994; Shober et al., 2006). In this work, we have chosen to combine XAS spectroscopic techniques with novel sampling strategies and data analysis approaches to identify soil P species, quantify their proportions, and determine the changes in P speciation. This work, along with conventional chemical extraction procedures, will improve the understanding of P chemistry in soils amended with both manures and inorganic P fertilizers.

A combination of wet chemical analysis and molecular analysis was used in this research to address the forms, nature, and transformation of P in manured, fertilized prairie soils from molecular scale to field scale over a time frame of weeks to years. It is hypothesized that different P species will be formed in the soil that will depend on amendment type, soil type, plant growth, rate, placement, and duration of application.

The overall objective of this Ph.D. dissertation was to assess the speciation of soil P in different manures and inorganic fertilizer-amended Saskatchewan soils as related to different amendment management practices and conditions. The project focused on accurate identification of P minerals formed in the short-term and long-term following the application of different manure and inorganic fertilizers (mono-ammonium phosphate) to two calcareous prairie soils (Brown and Black Chernozem) as affected by time, presence and absence of plants, landscape position, pH, and soil constituents. Manures evaluated in this study were solid cattle manure (SCM) and liquid hog manure (LHM), which are most commonly applied ones in the Canadian prairies. Management practices evaluated were rates of application and placement in the soil.

This dissertation is organized into seven chapters. Chapter 1 provides the context for the specific work along with a general hypothesis and objective. Chapter 2 reviews the literature addressing the overall forms and fate of soil P and appropriate methodologies for investigation. The next three chapters describe the experimental components to achieve the objective of this dissertation. Each chapter begins with a preface and contains a specific literature review to set the context for the work along with a complete materials and methods section. The sequence of chapters closely follows time scales of the study starting with very short-term (days to a month) to short-term periods (months to year) following amendment application, and ending with long-term (11 years) manure application history. Specifically, Chapter 3 covers the effects of manure application on P speciation and transformation in the presence and absence of plants growing under controlled environment conditions over a 5 wk period. In Chapter 4, the spatial distribution and chemical speciation of P in manure and inorganic P fertilizer banded soil is investigated in the months following application as affected by landscape, pH and soil constituents in the field. Chapter 5 describes a study to evaluate the specific P forms present in soil from long-term plots

that have received manure annually for one to eleven years. The synthesis of results and implications for future research are provided in Chapter 6 and the dissertation ends with a list of references in Chapter 7.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Introduction

Phosphorus is the eleventh-most common element of earth's crust (Flaten et al., 2003) and an essential macronutrient for plant growth and development (Havlin et al., 2005). It is a vital component of building blocks of genes and chromosomes and necessary for storage of energy through photosynthesis, transfer through cell membranes and the biosynthesis of proteins (Havlin et al., 2005). The amount of P present in soil solution (~ 0.05 mg g^{-1}) and the external applications of P, typically via inorganic fertilizers and manures (Chang et al., 2005), strongly affect agricultural production by modifying the availability of P to plants and environmental health by regulating retention, release, and transport of P. Inorganic P fertilizers are typically applied to soil as orthophosphate salts, which rapidly dissolve in soil solution and become readily available to plants. Phosphorus is present in organic amendments like animal manure in both inorganic and organic forms; the organic P can transform into the inorganic form of orthophosphate by mineralization processes that are dependent upon soil and environment conditions such as moisture, temperature, and pH over time (Khatiwada, 2011).

2.2 Phosphorus Fertilizer Sources

2.2.1 Inorganic sources

There are several commercial P fertilizer sources. In commercial fertilizer, P is generally expressed as P_2O_5 instead of elemental P (to convert to a P basis the P_2O_5 is multiplied by 0.43). Most of the inorganic P fertilizers are made from treating rock phosphate with acid (Havlin et al., 2005). Following application to soil, the inorganic fertilizer P granule is dissolved in soil solution rapidly and becomes readily available to plants. There are several types of commercial phosphate

fertilizers available in the market. The commonly used commercial P fertilizers for crop production in North America are given in Table 2.1. Mono-ammonium phosphate (MAP) is the dominant P fertilizer used in western Canada and Saskatchewan (Saskatchewan Ministry of Agriculture, 2006).

Table 2.1. The commonly used inorganic P fertilizer sources in North America.

Fertilizer	Formula	Symbol
Single super phosphate (0-20-0)	$Ca(H_2PO_4)_2.H_2O$	SSP
Triple Super Phosphate (0-46-0)	$Ca(HPO_4).H_2O$	TSP
Di-ammonium phosphate (18-46-0)	$(NH_4)_2HPO_4$	DAP
Mono-ammonium Phosphate (11-52-0)	$(NH_4)H_2PO_4$	MAP
Ammonium polyphosphate (10-34-0)	$(NH_4)_3HP_2O_7$	APP

2.2.2 Organic sources

Manure, biosolids, and crop residues are important organic sources of P that have been widely used for long periods of time before commercial inorganic P fertilizers became available in the 1900's. Phosphorus is present in organic sources in both inorganic and organic forms. For example, the inorganic forms of P account for 45% to 70% of total manure P in hog and cattle manure, and the rest of the total P is in organic P form (Zhang et al., 2002). In agricultural systems of the Great Plains of North America, manure is the main organic source of P that is land applied for crop production (Campbell et al., 1986; Chase et al., 1991).

2.3 The Phosphorus Cycle in Soil

The P cycle in the soil system is shown in Figure 2.1. Weathering of P bearing minerals in the parent material contributes to available P while the main external inputs of P in soil are inorganic fertilizers, crop residues, organic by-products, and manure. Mineralization-immobilization process can cycle P through organic and inorganic forms via microbial activity

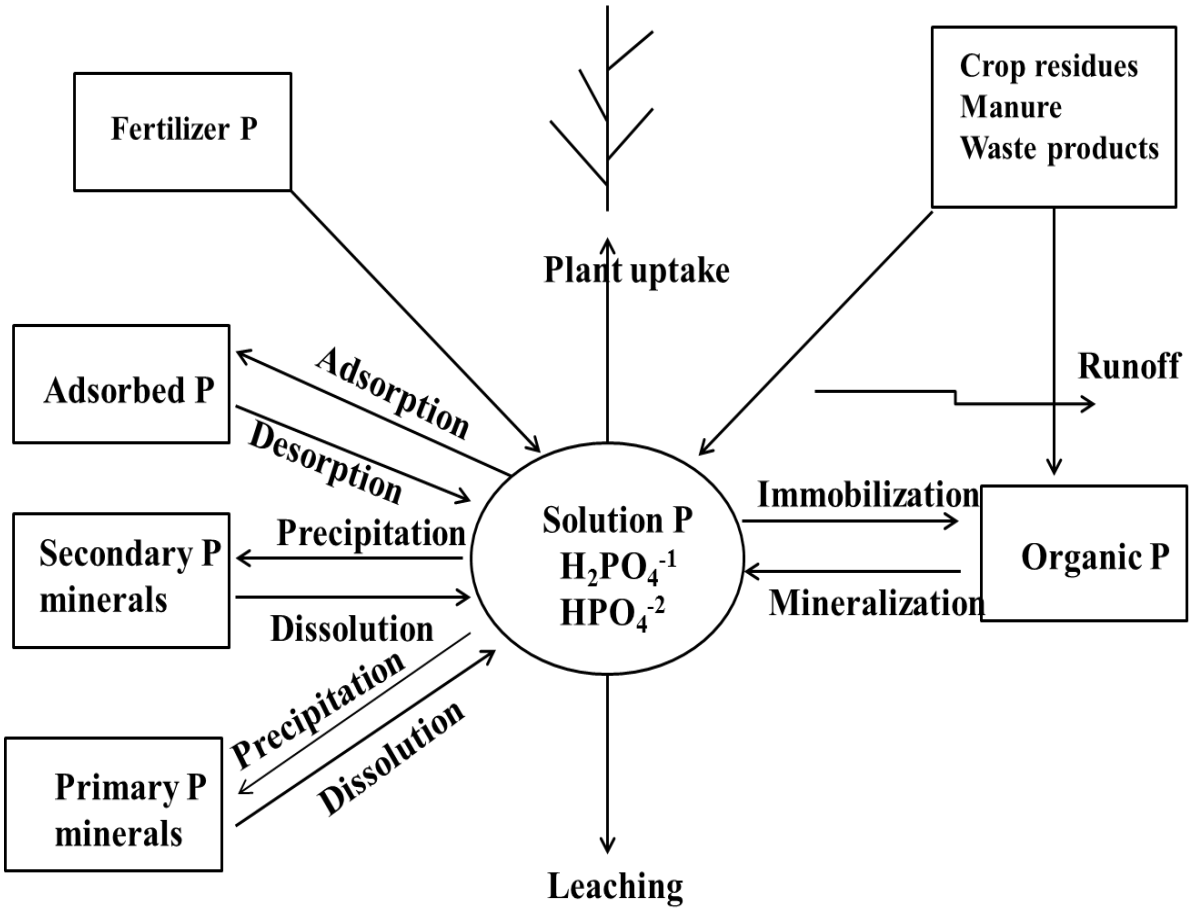

Figure 2.1. Simplified diagram of the phosphorus cycle in the soil system. Adapted from Havlin et al., 2005.

and the action of enzymes like phosphatases produced by plant roots. Plants can take up a portion of applied P operationally termed “labile”. Some portion of the applied P undergoes a series of chemical processes that can fix P into less soluble (non-labile) forms, and some portion may be lost from soil through different processes like crop removal, runoff, and erosion or leaching. The mobilization of labile P from agricultural field to surface water bodies is of great environmental concern. Application of inorganic and organic P fertilizer in excess of crop requirements often results in higher buildup of total and labile P in surface soil (Chang et al., 2005). The excess P can be lost from the soil to nearby water bodies, which is often the major

cause of eutrophication processes in aquatic systems (Sharpley et al., 1996; Maguire et al., 2000; Leinweber et al., 2002). Hence, to manage the fate of applied P and reduce potential loss of P from soils, the P cycle needs to be fully understood.

2.3.1 Soil phosphorus forms

Phosphorus exists in soil solution and in the solid phase in various inorganic and organic forms. All of the forms are not directly available to plants and the availability of P mainly depends greatly on type of mineral that is present and also the soil pH.

Available forms of P are present in soil solution as $H_2PO_4^{-1}$ and HPO_4^{-2} ions. The preponderance of one or the other of these forms in soil solution depends on soil solution pH (Havlin et al., 2005). The HPO_4^{-2} is the dominant ion above pH 7 (alkaline soil) and $H_2PO_4^{-1}$ ion increases in solution below neutrality (acidic soil) and becomes dominant with decreasing pH (Havlin et al., 2005). The relationship between the ionic form of P and pH is shown in Figure 2.2. The amount of soil solution P is low (< 1 ppm) in most soils as many of the phosphate compounds have low water solubility. The average P concentration in soil solution is ~ 0.05 ppm, and the soil solution P concentration required by plants varies from about 0.003 to 0.3 ppm (Havlin et al., 2005).

The inorganic P may be present in soils as mineral P forms and P forms adsorbed to surfaces. Inorganic orthophosphate reacts readily with soil constituents and undergoes precipitation or adsorption reactions. The presence of aluminum (Al), iron (Fe), and their oxides, and calcium carbonate ($CaCO_3$) are major controllers of P adsorption and precipitation in the soil system (McDonald, 2000). In acidic soils, Al and Fe are the dominant cations which react with phosphate. Phosphate is adsorbed to the surface of Fe and Al oxides and clay minerals or else is precipitated as secondary Fe and Al-P minerals.

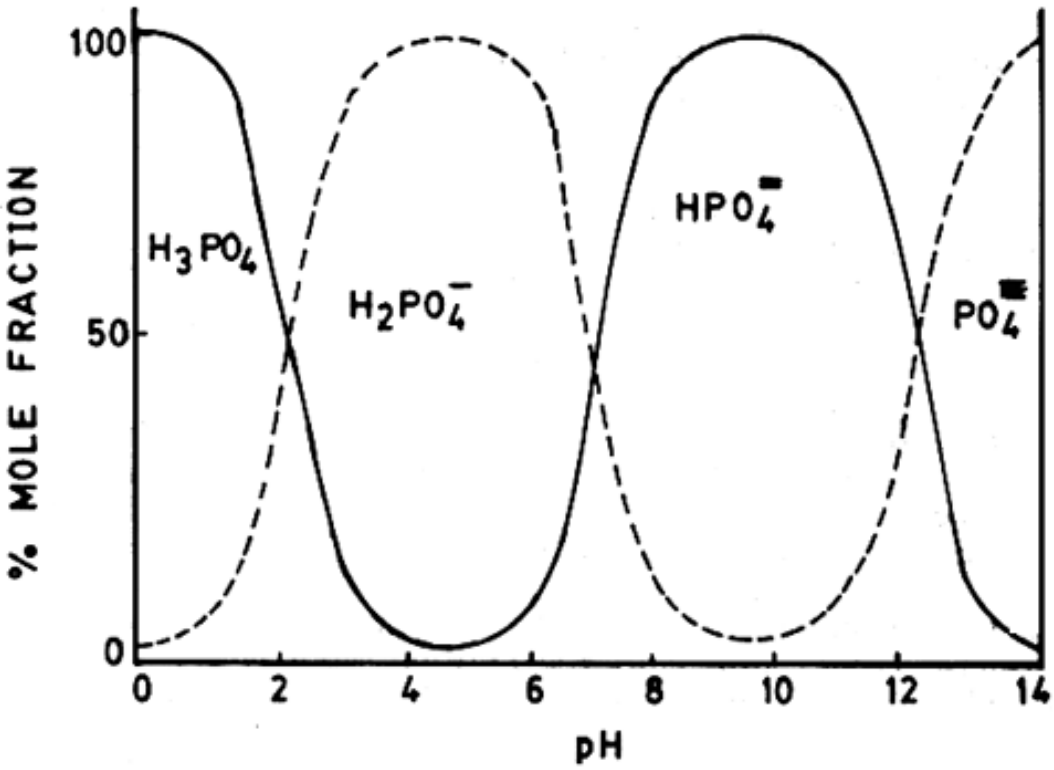

Figure 2.2. Relationship between soil solution pH and the relative concentration of different phosphate ions. Adapted from Havlin et al. (2005)

However, in neutral to calcareous soils, calcium is the dominant cation and P is adsorbed to surfaces of calcium carbonate and clay minerals or precipitated as secondary calcium phosphate (Ca-P) minerals (Sato et al., 2005). The P that is associated with Fe or Al is considered moderately labile when orthophosphate is bonded through one Al-O-P or Fe-O-P bond, while the slowly available forms are associated with the calcium (Ca) or magnesium (Mg). The common P minerals found in acid, neutral and calcareous soils are presented in Table 2.2.

Organic P represents about 50 % of the total P in soil (Havlin et al., 2005), and over a growing season less than 5 % of this is mineralized from stable organic complexes, crop residue decay, and applied organic fertilizers and made available for plant uptake (Daniel et al., 1994; Stumborg and Schoeanu, 2008). Soil organic P increases with increasing organic matter and

most organic P compounds are esters of orthophosphate including inositol phosphates, phospholipids, and nucleic acids (Pierzynski et al., 2000; Havlin et al., 2005).

Phosphatase enzymes produced by microbes and plant roots can cleave orthophosphate esters. Inorganic P also is transformed to organic P form in soil solution via immobilization process and both mineralization and immobilization processes can occur simultaneously and continuously in soils (Flaten et al., 2003).

Table 2.2. Common P minerals found in acid, neutral, and calcareous soils. Adapted from Havlin et al. (2005)

Minerals	Formula
Acid soils	
Variscite	$AlPO_4.2H_2O$
Strengite	$FePO_4.2H_2O$
Neutral and calcareous soils	
Dicalcium phosphate dihydrate	$CaHPO_4.2H_2O$
Dicalcium phosphate	$CaHPO_4$
Struvite	$NH_4MgPO_4 \cdot 6H_2O$
Octacalcium phosphate	$Ca_4H(PO_4)_3.2.5H_2O$
b-tricalcium phosphate	$Ca_3(PO4)_3$
Hydroxyapatite	$Ca_5(PO4)_3OH$
Fluorapatite	$Ca_5(PO_4)_3F$

*Minerals are listed in order of decreasing solubility

2.4 **Fate of Phosphorus in Soil**

Phosphorus is applied to soils as inorganic and organic fertilizers. However, plants do not have access to all the P that is applied to the soil. Over the time, plants will assimilate part of the available P. The remaining of P in the soil solution that the plant cannot uptake reacts with other soil components, and is adsorbed to the soil mineral surfaces or precipitated as secondary P compounds or else immobilized by microorganisms (Ghosh et al., 1996; Hansen et al., 2002). Phosphorus is released in soil solution through weathering of soil minerals, desorption of

adsorbed P from soil surfaces, dissolution of primary and secondary P minerals, and mineralization of organic forms of P (Figure 2.1).

Adsorption is the surface phenomenon that accumulates liquid or gaseous materials on the surface of a solid (Larsen, 1967). In spite of net negative surface charge, soil particles are still capable of strongly retaining P oxyanions and reducing the mobility and thus the availability of P following adsorption. In soil, the main adsorbents of P ions are Fe and Al oxides and hydrous oxides, alumino-silicate clay minerals, carbonates and soil organic matter. In acidic soil, Fe and Al-oxides exist as discrete particles, and as a surface layer on other clay mineral particles. These oxides are hydroxylated in the presence of water forming the positively charged surfaces that can adsorb P anions. This phenomenon is controlled by the pH of the medium. The low pH in acidic soil dominates the adsorption through the formation of Al-O-P or Fe-O-P bonds. In addition, there is an increase in P adsorption with the increase in clay content that has large surface area (Ryan et al., 1985). Though the soil organic matter is normally negatively charged, it can adsorb P in association with common cations such as Fe^{3+}, Al^{3+} or Ca^{2+} (Holford and Mattingly, 1975). In calcareous soil, $CaCO_3$ adsorbs P from solution (Griffin and Jurinak, 1974) by replacing CO_3^{-2} on the $CaCO_3$ surface, but this bonding is much less strong than the P adsorbed on Fe-oxides.

Precipitation is a chemical process that describes the formation of discrete, solid phases from soluble ions in the soil solution. In high soil pH, P reacts with Ca or Mg to form Ca or Mg-phosphates, while at low soil pH, P reacts with Fe and Al. Those reactions that form solid phase precipitates greatly reduce the mobility and the availability of P. In calcareous soil, Ca is the dominant cation to react with inorganic P and forms sparingly soluble Ca-P minerals like dicalcium phosphate (i.e. brushite), octacalcium phosphate, and tricalcium phosphate within a few days to few months. Ultimately hydroxyapatite (HA) is formed, the least soluble Ca-P

compound (Lindsay, 1979; Sample et al., 1980; Freeman and Rowell, 1981; Havlin et al., 2005). However, in acid soil, Al and Fe are the dominant cations to react with inorganic P and precipitate as $AlPO_4$ and $FePO_4$ compounds.

As the P fixation (adsorption and precipitation) processes largely depend on soil pH (Havlin et al., 2005), the modification of pH may increase or decrease the availability and mobility of certain kinds of fixed P (Fig. 2.3). For example, increasing pH (such as by applying lime in acid soil) increases the solubility of Fe-P and Al-P and increases the availability of $H_2PO_4^-$ ion in soil solution. However, with the increase of pH above neutrality, Ca-P precipitates and reduces the mobility and availability of soil P. Long-term cropping history that increases soil organic matter can also modify the soil P availability by affecting sorption and mineralization.

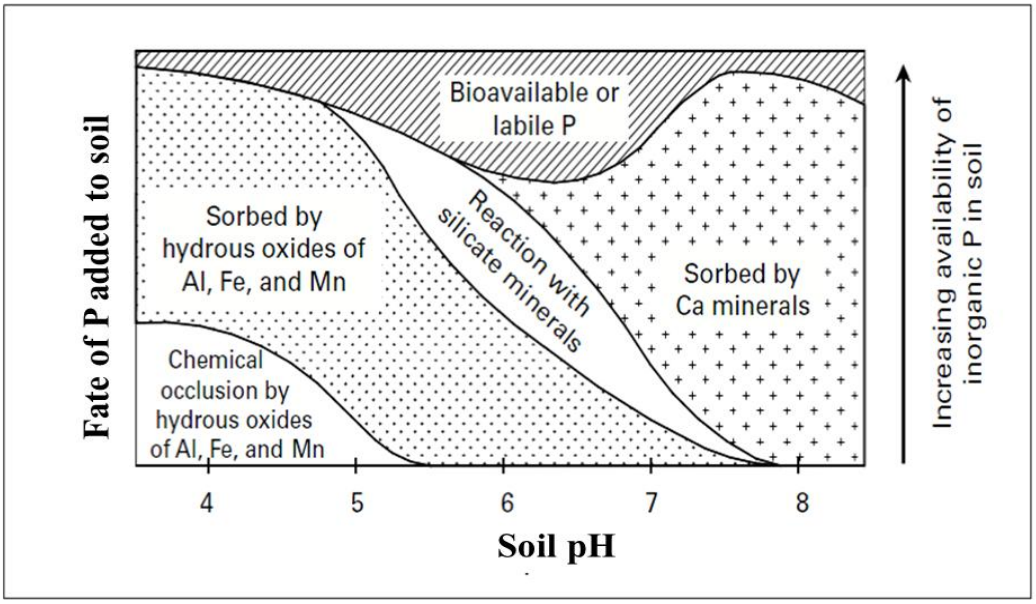

Figure 2.3. Approximate representation of the fate of phosphorus added to soil by sorption and occlusion in inorganic forms, as a function of pH. Adapted from Sharpley et al. (1984)

Manure P reacts differently when added to soil compared to inorganic P fertilizer. The low concentration of P in manure cannot create a localized zone of high P concentration and

ionic strength. This may result in reduced dissolution of soil minerals and decreased precipitation as P compounds.

Depending on the class of livestock and the feed mineral supplements, manure can contain substantial quantities of cations such as Ca, Mg, K, Na which may react with P or displace other cations from the soil solution and exchange to react with P. Organic acids from the manures have been shown to reduce both P sorption (Ohno and Crannell, 1996) and precipitation of Ca-P (Grossl and Inskeep, 1991), but can increase the precipitation of Al-P minerals (Traina et al., 1986). However, adsorption-precipitation reactions may occur simultaneously in soil system though it has been reported that high P concentration favours precipitation and low P concentration favours adsorption process (Havlin et al., 2005; Tunesi et al., 1999). A pH range of 5.5 to 7 is considered to be the most suitable for maximizing available P in soil solution (Brady and Well, 2002).

Immobilization is the conversion of an element from the inorganic to the organic form, usually in soil microbial tissue. This depends on the relative ratios of C, N, and P in the soil, the form of P in the amendment and the moisture and temperature regime that controls biological growth. A C:P ratio > 300 favours the P immobilization, while a ratio < 200 favours mineralization (Douglas and Albrecht, 2000). Application of high amount of inorganic P fertilizer such as mono-ammonium phosphate along with a carbon source such as wheat straw will support immobilization. Manure application increases net mineralization by decreasing soil C:P ratio and increasing the content of mineralizable organic P. Organic acids also slow down the formation of more stable Ca-P minerals in soil such as octacalcium phosphate (Grossl and Inskeep, 1991) and hydroxyapatite (Inskeep and Silvertooth, 1988; Grossl and Inskeep, 1992).

The behavior of inorganic and organic P fertilizers in soil is likely to vary as a consequence of manure and soil conditions in prairie soils along with management practices. The study of reaction products of manure P in comparison to fertilizer P can help us understand the fate of manure P in calcareous prairie soils and help to design better manure P management practices.

2.5 Losses of Phosphorus from Soil

Phosphorus losses from soil to surface water can occur by surface runoff, erosion, and/or leaching. Phosphorus losses in runoff occur primarily during rainfall interaction with the top 1- to 2.5 cm of soil, causing desorption from crop residues, soil and fertilizer sources, and movement off-site in solution (Sharpley, 1985). Phosphorus is lost via runoff as dissolved inorganic and organic forms (Daniel et al., 1994) and the risk of P loss in runoff increases as STP concentration increases near the soil surface (Pote et al., 1996). Orthophosphate is the soluble, inorganic form of P in runoff water, which is readily available for algal and plant uptake (Sharpley et al., 1992) and when P concentration is higher than 0.02 mg P L^{-1} and reaches 0.1 mg total P L^{-1} in water, there is increased risk of eutrophication (Correll, 1998).

Phosphorus losses via leaching occur when soils have low P fixing capacity along with high water infiltration rates that may occur under irrigation or heavy rainfall events. Phosphorus can be leached as dissolved inorganic (Djodjic et al., 2003) and organic forms (Chardon et al., 1997). The P leaching loss from fertilizer applied soil is mainly dependent on soil P levels, soil P-retention capacities, and P sources (Kleinman et al., 2003). Leaching loss of P from continuous manure applications made to soil poses potential environmental risk if the labile P content is elevated (Shadi et al., 2010).

Erosion can cause significant P loss through particle movement into nearby surface waters, as P bound to soil and organic matter accounts for a large proportion of total soil P. The loss as particulate P forms to nearby surface water varies with the total concentration of P in the soil and the size of the particle. Smaller mineral particles (e.g., clay) and organic matter that usually contain high content of P can move easily and contribute to significant losses (Nelson and Logan, 1983). Generally, particulate P losses from fine-textured soils (e.g., clay or clay loam) will be greater than those from coarse textured soils because fine-textured soils have colloidal clays that are easily transported with water and higher sorption capacity than coarse textured soils. To minimize the potential environmental risk from P loss, it is important to assess soil P levels and P forms over time, and apply management practices that reduce P loss especially through runoff.

2.6 Selection of Analytical Methods for Assessing Soil Phosphorus

Phosphorus is applied to agricultural lands for crop production. Soil P buildup with repeated application of manure or inorganic fertilizer increases the risk of P losses to surface and ground water and causes eutrophication (Sharpley et al., 1994; Sharpley and Withers, 1994; Sims et al., 1998). Hence, it is important to measure soil total, available (soil test P), and ideally, the specific chemical forms of P in soil. This is important for assessing P fertilizer requirements as well as environment risk. There are several analytical methods that are used to determine total, STP, and different forms (speciation of P) in soils. Selection of a suitable method is based on its efficiency, required criteria, availability of resources, and its ability to measure what is significant in the biological, chemical, and physical realm of soil P cycling (Khatiwada, 2011).

2.6.1. Soil total phosphorus

The total P content of soil can vary from 100 to 2500 mg P kg^{-1}, and mainly depends on the soil parent material, texture, and different P management practices used (Daniel et al., 1994). There are several methods that are used to determine total P in soil (Bray and Kurtz, 1945; Muir, 1952; Jackson, 1958; Thomas et al., 1967; Syers et al., 1968; Sommers and Nelson, 1972; Dick and Tabatabai, 1977; Bremner and Mulvaney, 1982; Olsen and Sommers, 1982; Bowman, 1988). The standard acid digestion method described by Thomas et al. (1967) is widely used for total P analysis in manure and manure amended soil (Qian et al., 2000; Stumborg and Schoenau, 2008; Kar et al., 2011). This method is suitable for the analysis of a small number of samples and gives better results compared to the Na_2CO_3 fusion method (Thomas et al., 1967). Gasparatos and Haidouti (2001) reported that the amounts of total P measured by this method in 15 different soils were 95% to 100% of those extracted with $HClO_4$ digestion. In the Thomas et al. (1967) method, approximately 0.25 g soil is placed in a glass digestion tube and is digested with 5 mL of concentrated H_2SO_4. The soil acid suspension is digested for 30 min at 360 ˚C in a block heater. The tubes are then removed from the heater and cooled for 30 minutes at room temperature, and then 0.5 mL H_2O_2 is added to breakdown the organic matter, and the soil-acid mixture is vortexed. This digestion process is repeated a total of 8 to 10 times. Finally, samples are allowed to cool for 30 minutes, and enough deionized water is added to reach a volume of 75 mL. The digested samples are collected and analyzed for total P concentration. The P recovery in this method is typically 95% or better.

2.6.2 Available phosphorus level

Several STP methods are used to assess plant available P status in soils to predict crop yield responses to added P fertilizer and also to assess the environmental risk imposed by high STP level in soils (Kashem et al., 2004; Kumaragame et al., 2007). Several researchers (Pote et

al., 1999; Pierson et al., 2001; Fang et al., 2002; Torbert et al., 2002) have shown that there is direct relationship between STP and P concentrations in overland water flow from both non-calcareous and calcareous soils, which is useful for environmental purposes. Soil test P methods are divided into agronomic soil test and environmental test methods. Soil test P methods are used for agronomic purposes such as measurement of available soil P concentration for predicting plant uptake and additional fertilizer P requirements, and for environmental purposes for predicting the mobile P fraction in soil. Agronomic test methods are widely verified and their suitability for use in soils with various physical and chemical properties is well documented (Smillie and Syers, 1972; Kamprath and Watson, 1980; Menon et al., 1991; Beegle, 2005). Agronomic test methods such as sodium bicarbonate extraction (Olsen et al., 1954), modified Kelowna (Qian et al., 1994), and resin extraction (Qian and Schoenau, 2002) are useful to estimate the labile available P to plants throughout the growing season (Olsen et al., 1954; Van Lierop, 1988; Wright et al., 2003). These methods have been shown to be well suited for neutral and calcareous soils and are widely used in western Canada (Havlin et al., 2005; Kumaragame et al., 2007; Stumborg and Schoenau, 2008). Environmental soil P test methods such as distilled water extraction (Schoenau and Huang, 1991) is used to estimate the mobile P fractions (Atia and Mallarino, 2002) that are subject to loss from the soil and have shown high correlation with dissolved P in runoff (Pote et al., 1996; McDowell and Sharpley, 2001). The water extraction method has been used to test soil labile P level for more than 30 years (Van der Paauw, 1971) and gives a good estimate of P loss potential (Wright et al., 2003).

There are various methods that are currently being used to measure soil labile P levels in western Canada. The selection of the most accurate STP method is very important to determine soil labile P levels for both agronomic and environmental purposes under inorganic or organic

fertilizer management practices in calcareous Canadian Prairie soils. The above discussed STP methods have been previously shown to be significantly correlated with each other and to plant uptake and concentration in runoff water (Pote et al., 1996; Sims et al., 2000; Kashem et al., 2004; Stumborg and Schoenau, 2008). Hence, any of these methods is suitable to measure labile P levels in manure amended or unamended calcareous soils and can be used for P determination in soils in order to improve P fertilizer recommendations.

2.6.3 Chemical speciation of phosphorus

The trends in bioavailability of P in amended soils over time depend on the transformation and redistribution of P among various labile and stable forms. Phosphorus may enter into soil solution in on available form directly from inorganic or organic fertilizers and from soil solid phase through desorption, dissolution, and mineralization or can be transformed to unavailable forms through adsorption, precipitation, and immobilization processes. Hence, knowledge of the solid phase P species in soils amended with manure is vital in developing better manure P management practices, as the transformation and fate of different P species in soil is controlled by its chemical forms.

2.6.3.1 Sequential chemical extractions

The sequential chemical P fractionation procedure developed by Hedley et al. (1982) and later modified by Tiessen and Moir (1994) is widely used to partition different forms of P in manures (Nair et al., 1995; Gale et al., 2000; Sharpley and Moyer, 2000; Ajiboye et al., 2004; Akhtar et al., 2005) and manure amended soils (Sui et al., 1999; He et al., 2004; Kashem et al., 2004; Qian et al., 2004; Lehmann et al., 2005; Sato et al., 2005). This procedure has been a useful tool in separating different soil P forms and following the fate of added P in soil as

affected by soil management and crop rotation practices (O'Halloran et al., 1987; McKenzie et al., 1992). This procedure provides a rapid estimate of operationally defined pools of P; e.g. bioavailable P, labile and/or moderately labile, stable inorganic and organic P, and more stable residual P. These operationally defined fractions are based upon P extractability with progressively stronger extractants such as water or ion exchange resin, followed by $NaHCO_3$, NaOH, HCl, and H_2SO_4 extractants (Sharpley and Moyer, 2000; Seiter et al., 2008), respectively. Phosphorus extracted via resin or water and $NaHCO_3$ is considered as "readily available" labile P (Schoenau et al., 1989), NaOH extractable P is considered as "moderately labile" (Hedley et al., 1982) as this removes both Fe-oxide and Al-oxide associated P (Bowman and Cole, 1978) and can contribute to plant-available soil P pools (Tiessen et al., 1984), HCl extractable P is considered to be largely stable calcium phosphate that is less available to plants, and H_2SO_4 digestible P is considered the stable residual P fraction (Hedley et al., 1982). This chemical extraction procedure sequentially characterizes the different P forms based on their solubility but cannot provide specific information about the exact chemical species of P in the soils. Therefore, X-ray based molecular-scale analysis techniques have been used to identify different P species present in soils (Hesterberg et al., 1999; Beauchemin et al., 2003; Shober et al., 2006; Ajiboye et al., 2008; Kruse and Leinweber, 2008). Solid-state speciation of P using molecular-scale analysis techniques in conjunction with sequential chemical extraction can improve our understanding of the forms, transformation, and fate of P in P amended agricultural soils. Different spectral techniques are being used for speciation of organic and inorganic forms of P in manures, sediments, biosolids, and commercial fertilizers. These methods include X-ray diffraction (XRD), nuclear magnetic resonance (NMR), and X-ray absorption (XAS) spectroscopy.

2.6.3.2 X-ray diffraction

X-ray diffraction is a versatile, nondestructive, and rapid analytical technique that is used in the characterization and identification of crystalline materials in soils and sediments (Vogel and Sparks, 2010). This method has long been used by many researchers (Fordham and Schwertmann, 1977; Bril and Salomons, 1990; Shand et al., 2005; Toor et al., 2006; Gungor et al., 2007) to identify P minerals in soils, manures, and poultry litter. CuiHong et al. (2010) used XRD technique to identify different P species present in sediment samples collected from Lake Hongfeng in China and found that $AlPO_4$ was present at relatively high concentration in all sediment samples. Shand et al. (2005) used XRD to quantitatively identify P minerals in sheep feces and showed that dicalcium phosphate dehydrate (DCPD) and struvite accounted for 63% of total P. Gungor et al. (2007) also used XRD to identify P minerals present in dairy manure and struvite was unambiguously identified in both the raw and anaerobically digested dairy manure samples. However, identification of different mineral species in soil and manure using XRD technique is limited because P minerals are not in as high concentration in bulk soil and amorphous P minerals cannot be identified by XRD (Khatiwada, 2011). Identification of P minerals in P fertilized soil was not successful due to lower concentration of P minerals in soils as well as their poor crystallinity (Pierzynski et al., 1990; Ippolito et al., 2003; Gungor et al., 2007). This method can be used in conjunction with other direct methods of P speciation that extract and concentrate P for better interpretation of results. Several researchers (Pierzynski et al., 1990; Gungor et al., 2007; CuiHong et al., 2010) have used XRD in combination with other direct speciation methods to successfully identify different P species in soils, sediments, and manures.

2.6.3.3 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy has been a useful technique to obtain

molecular-scale information on P species in soils (Adams and Byrne, 1989; Magid et al., 1996; Condron et al., 1997), organic P in manures (Hunger et al., 2004; Maguire et al., 2004; Toor et al., 2006), composts (Frossard et al., 1994), and biosolids (Hinedi et al., 1989). This technique can be used to analyze either solution or solid state samples. Hinedi et al. (1989) used solid-state NMR spectroscopy and found that calcium phosphate, aluminum phosphate, and pyrophosphate solid phases were present in anaerobically digested biosolids and biosolids-amended soils. These authors also used solution NMR spectroscopy to characterize organic forms of P in dairy manure. Hunger et al. (2004) used solid-state ^{31}P NMR spectroscopy to identify different residue P species present after each step of sequential extraction in poultry manure samples. Turner and Leytem (2004) used solution state NMR spectroscopy to investigate P species in poultry manure samples after each chemical extraction step. Hence, the NMR spectroscopy has proven to be a powerful tool for characterization of solid state P speciation.

The NMR technique is widely used for study of speciation and transformation of organic P compounds in soil and organic byproducts (Toor et al., 2006). In contrast, the major limitation of the NMR spectroscopic technique, is its limited ability to identify specific inorganic P minerals such as Ca-P, Fe-P, and Al-P minerals in soils (Dou et al., 2002; Turner, 2004; Negassa and Kruse, 2010) that mainly control the P solubility in environmental media (Toor et al., 2006). This is because the presence of paramagnetic cations like Fe, Al, and Mn associated with P interact with the external magnetic field and decrease the resolution of the NMR spectra (Hunger et al., 2004; Kizewski et al., 2010). Another limitation to use of NMR techniques is the pretreatment of samples required. Therefore, an *in situ* solid-state speciation technique is required. X-ray absorption spectroscopy is advantageous over NMR spectroscopy because XAS techniques show better sensitivity, are an *in situ* (no pretreatment of samples required)

assessment, and can be performed with every element (Toor et al., 2006). For example, X-ray absorption near-edge structure spectroscopy cannot only identify P species, but also Ca. This is useful as Ca is abundant in poultry litter and plays an important role in characterizing P minerals in soils and litter (Moore et al., 1995; Jackson et al., 2003; Toor et al., 2005).

2.6.3.4 X-ray absorption spectroscopy

X-ray absorption spectroscopy (XAS) is an analytical tool that has been successfully used for speciation of elements in soil, sediments, and environmental samples. X-ray absorption spectroscopy is a nondestructive (without additional sample treatment) element-specific technique (Beauchemin et al., 2003) and is able to determine the oxidation states, local chemical and structural state of an element (Fendorf and Sparks, 1996). X-ray absorption spectroscopy comprises X-ray absorption near-edge structure (XANES) spectroscopy and extended X-ray absorption fine structure (EXAFS) spectroscopy. The physical basis of both XANES and EXAFS is the same but XANES is sensitive to formal oxidation state and local molecular bonding environment of the element, while EXAFS is used to determine the distances, coordination number, and of the neighbor elements of the absorbing atom (Fendorf and Sparks, 1996).

X-ray absorption near-edge structure spectroscopy has been used for speciation of P in soils (Hesterberg et al., 1999; Beauchemin et al., 2003; Shober et al., 2006; Ajiboye et al., 2008; Kruse and Leinweber, 2008; Peak et al., 2012), manure and biosolids (Peak et al., 2002; Toor et al., 2005; Maguire et al., 2006; Kar et al., 2011), and manure amended soils (Beauchemin et al., 2003; Ajiboye et al., 2008). For example, P K-edge XANES spectroscopic results revealed that long-term application of inorganic P fertilizer resulted in the formation of adsorbed and crystalline apatite type P forms whereas a mixture of aluminum and iron phosphates and poorly

crystalline dicalcium phosphate (DCP) were formed after biosolids application (Kar et al., 2011). Sato et al. (2005) reported that P was associated with Fe and soluble Ca-P species in the short-term, and a more stable form of β-tricalcium phosphate species was formed over longer time periods in poultry manure amended acidic forest soils using XANES spectroscopy. Speciation of P using XANES spectroscopy has received less attention in agricultural soils, but can potentially provide a better approach for predicting plant availability, mobilization, transformation, and potential off-site transport of P in agricultural lands.

X-ray absorption spectroscopy is based on synchrotron radiation, which is extremely bright electromagnetic radiation. Electrons radiate electromagnetic radiation in the direction that they were traveling when they are accelerated by a force perpendicular to their velocity (Elder et al., 1947; Sham and Rivers, 2002) at relativistic speeds (i.e., approximately the speed of light). In the modern synchrotron facilities, a linear accelerator produces and accelerates electrons and transports to the second accelerator booster ring to accelerate electrons at nearly the speed of light (Winick, 1994). These electrons are then transferred to the storage ring. The storage ring is polyhedral and highly focused electromagnetic radiation is emitted from this ring which is used for experimental purposes. The bend magnets fitted at each corner of the storage ring maintain the acceleration of electrons and direct them to the next straight section.

The insertion devices, known as wigglers and undulators, are installed into the storage ring between the bending magnets to increase the flux of produced synchrotron radiation. The selection of necessary energy range is important to get better resolution. The selection of energy of the radiation is accomplished by passing the high intensity x-ray energy through a monochromator, a device that selects the energy of the radiation that is delivered to the sample. The monochromator is diffraction-based and determines the wavelength of passing energy

through this device according to the Bragg's law ($n\lambda = 2d \sin \theta$). The data collection and sample insertion is accomplished at the endstation which is application-specific. The produced synchrotron radiation is directed to the experimental stations where the desired wavelengths are selected for different kinds of experiments. The desired wavelengths (λ) for XAS experiments are in the X-ray region (0.01-10 nm) and expressed as energy, E (electron volt, eV) according to the following equation:

$$E=hc/\lambda$$

Where h is Planck's constant and c, the speed of light.

The explanation of XAS principles and theory can be found in the literature (Fendorf and Sparks, 1996) and on the websites of many synchrotron facilities (www.xafs.org, 2003; Lightsources.org, 2007). The X-ray is electromagnetic radiation with energies ranging from ~ 50 eV to 500 keV and X-ray light is absorbed by all matter through the photo-electric effect (Newville, 2003). When an X-ray photon is absorbed by an atom at certain energies, a quantum core level (K, L, or M) electron of the atom is ejected from the central atom into the continuum leaving the atom in an excited state with an empty electronic level. The ejected electron with excess energy obtained from the x-ray is called a photoelectron (Toor et al., 2006). As the electron is ejected, the core hole is filled by decay of a higher energy level electron from the atom. When the electron fills the core hole, it releases excess energy in the form of X-ray fluorescence since this electron has higher energy than the core level. The intensity of the released fluorescence is measured over a range of incident photon energies to produce an XAS spectrum. The absorption edge at a photon energy produced by XAS spectrum occurs near the binding energy of core electrons at which core electrons of the element are ejected. The energy level of the electrons that the absorption edge occurs is unique and gives information about

oxidation states, and the local molecular bonding environment of the element. For example, the absorption edge corresponding to the energy necessary to eject electrons from the K shell (1s orbital) of P atoms is ~ 2150.0 eV (Toor et al., 2006).

The absorption spectrum is divided into the near edge (XANES) region and the extended (EXAFS) region that it has been mentioned earlier. The XANES region consists of ~20 eV before the absorption edge (white line energy) to ~100 eV past the edge and the region well above this edge is EXAFS region. The energy position of the absorption edge and the structure in the XANES region of XAFS provide information on local molecular coordination environment and symmetry of element of interests (Toor et al., 2006).

The XANES spectra can be analyzed both qualitatively and quantitatively. The qualitative analysis of XANES spectra is performed by comparing the spectra of the unknown sample with the spectral features of standard compounds as a fingerprint technique. The pre-edge peak intensity and position, white line energy position, and resonance features past the edge of P K-edge XANES spectra are affected by local chemical environment (Toor et al., 2006). These features are unique for each metal since XANES is element specific.

The P K-edge XANES spectra of various P reference compounds commonly used for fingerprinting technique are shown in Figure 2.4. The XANES spectra for Ca-P compounds show the shoulder at 2-10 eV past the white line peak. This intensity of the shoulder becomes more prominent with increasing crystallinity of the minerals like apatite and becomes less prominent with the increasing solubility such as dicalcium phosphate (Toor et al., 2006). Crystalline Al-bonded phosphate minerals have a peak around 5 eV past the white line energy (Toor et al., 2006). This peak is absent in the adsorbed phosphate on Al-oxide or hydroxides, and can be used to differentiate amorphous P minerals from crystalline (precipitated) phosphate

minerals such as variscite and wavelite. The strong pre-edge feature of Fe (III) phosphate minerals occurs 2-5 eV on the low energy side of white line. This feature is more prominent for strengite, less prominent for phosphosiderite, and absent for vivianite (Toor et al., 2006).

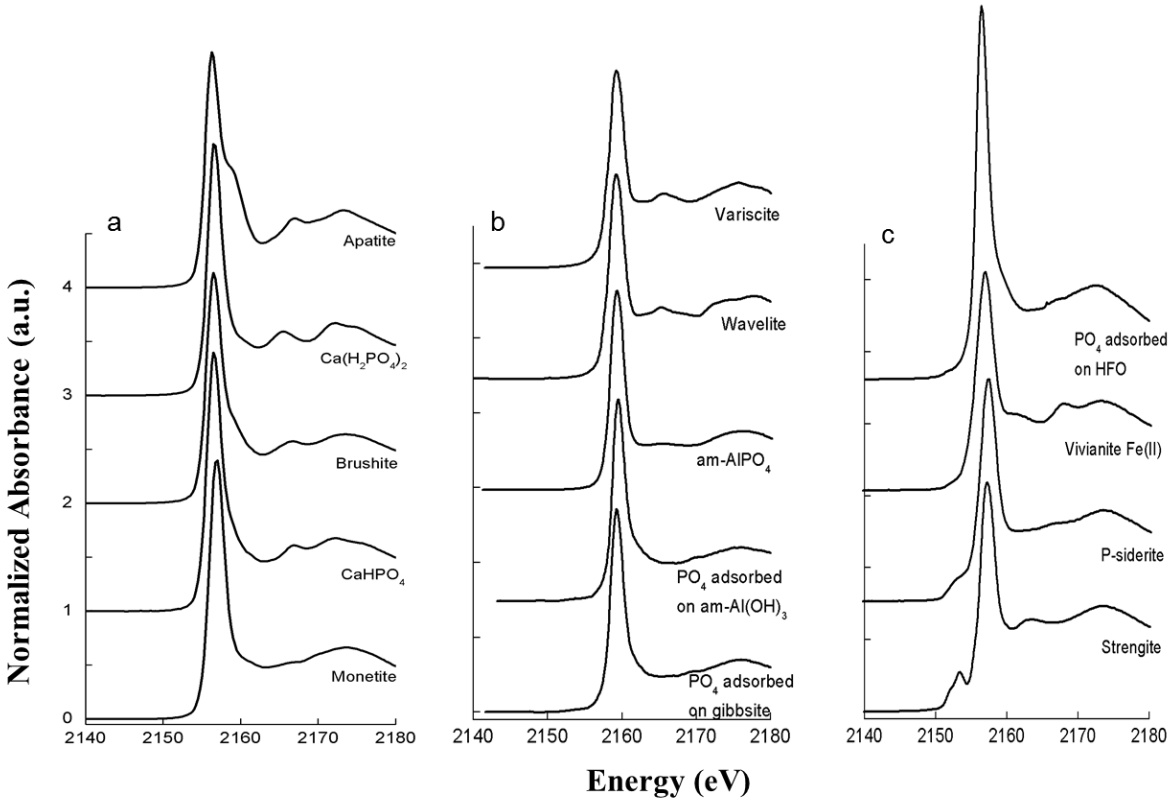

Figure 2.4. Normalized P K-edge XANES spectra of (a) $CaPO_4$, (b) $AlPO_4$, and (c) $FePO_4$ reference compounds. Adapted from Toor et al. (2006)

These spectral features also are used to distinguish Ca-phosphate minerals from Al- or Fe-phosphate minerals in a mixed system. Several researchers (Hesterberg et al., 1999; Ajiboye et al., 2008; Kruse et al., 2010; Kar et al., 2011) have used these spectral features to identify different P minerals in soils, manure, biosolids, poultry litter, agricultural byproducts, and amended soils. Sato et al. (2005) used this fingerprint technique and reported that DCP and Fe-P were formed over the short term in poultry manure amended forest soil. Kar et al. (2011) used fingerprint technique to analyze XANES data and found that adsorbed and crystalline apatite

forms of P were present in a long-term inorganic P fertilized soil, whereas a mixture of Al- and Fe-phosphates and poorly crystalline DCP minerals were formed in biosolids applied soil. Lombi et al. (2006) also used XANES spectroscopy and isotopic dilution method and reported that P precipitated as HA in the injection zone when mono-ammonium phosphate (MAP) was applied in granule form to a calcareous soil.

Quantitative XANES analysis is performed using the linear combination (LC) fitting approach to estimate the relative proportion of the species present in the sample (Ajiboye, 2008). This is performed by fitting the unknown sample spectrum with a group of reference compounds and finding the best combination fit. The LC fitting is based on the concept that an XAS spectrum represents the weighted average of signals from all atoms of the x-ray absorbing element (P in this case) as affected by their local molecular bonding environment (Toor et al., 2005). For LC fit, the averaged XANES spectra are background subtracted to the pre-edge region using a first-order polynomial fit and are normalized to an edge jump of 1.0. Linear combination fitting is then performed over the large spectral energy range (e.g., 2140-2180 eV for P) to include more chemical information and therefore increase the probability that similar standards can be properly distinguished (Toor et al., 2005; Peak et al., 2012). The initial E_0 value is allowed to vary to provide an initial estimate of percent contribution, but in the second iteration E_0 values are fixed to "0" for all components since the beamline is calibrated to a standard compound regularly during data collection. Several researchers (Peak et al., 2002; Beauchemin et al., 2003; Sato et al., 2005; Ajiboye, 2008) have applied LC fitting to quantitatively estimate the concentrations of P minerals in soils. Figure 2.5 shows LC fitting results of XANES spectra from the inorganically fertilized soil (A) and biosolids sample (B) (Peak et al., 2012). This figure shows that P was mainly present as adsorbed P (35%) and apatite forms (65%) in inorganically

fertilized soil while, dicalcium phosphate (29%) and organic P (15%) along with ferric phosphate (55%) were found in the biosolids applied soil. Beauchemin et al. (2003) quantitatively analyzed P minerals in soils and reported that applied ammonium phosphate fertilizer in soil transformed to hydroxyapatite and octa-calcium phosphates minerals, ranging from 11 to 59 and 24 to 53% of total P, respectively. The rest of the P was present as adsorbed P on aluminum hydroxide (18-27%), ferrihydrite (17-60%), goethite (15-23%), and alumina (16-34%).

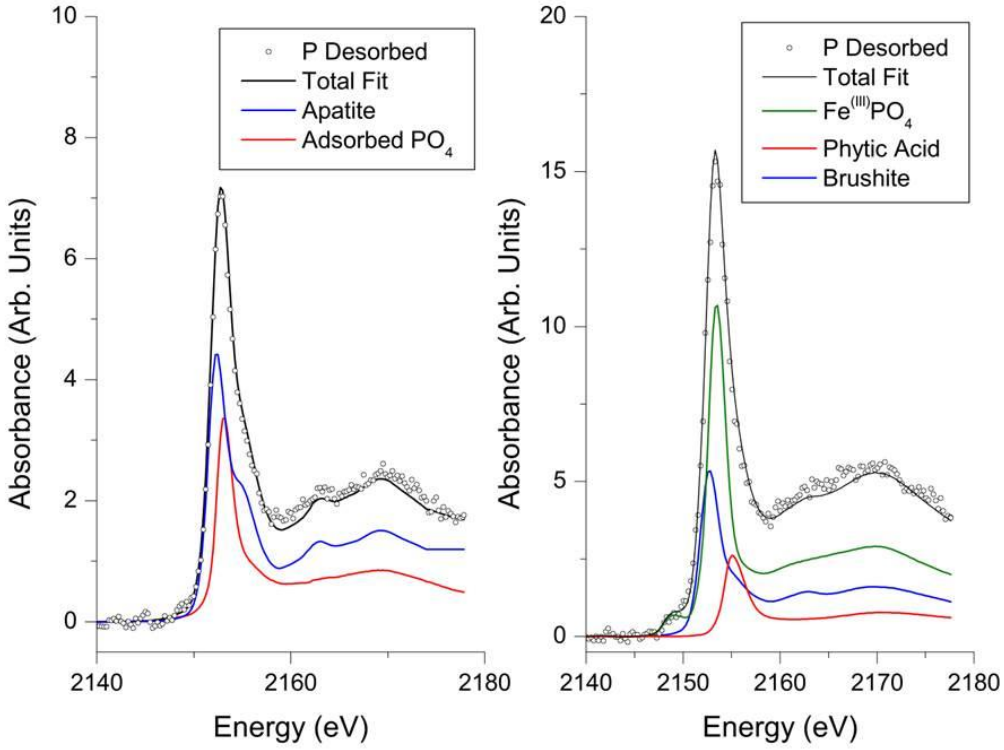

Figure 2.5. Linear combination XANES fit of differential XANES spectra from the inorganically fertilized soil (A) and biosolids sample (B). Adapted from Peak et al. (2012)

The XAFS data can be collected using three different detection modes: transmission, fluorescence yield (FY), and total electron yield (TEY) mode. Transmission mode (log I/I_0) is used to collect XAFS data at high photon energies (> 4000 eV) and for concentrated samples. A detector is directly placed before (I_0) and after (I) the sample and allows the x-ray beam to

completely pass through the sample (Toor et al., 2006) and then the intensity of the beam is measured. In hard x-ray experiments, the transmission data collection is not possible for the K-edge of P due to its very narrow penetration depth (only a few microns) (Toor et al., 2006). Instead, two other methods may be used. The FY is the preferred data collection mode for thin and lower concentrated samples. For experiments in FY mode, the sample needs to be diluted to the same concentration with a nonabsorbing powder such as BN_3 (Toor et al., 2006) and finely ground. The fluorescence spectra can be collected either as a total fluorescence yield or in partial fluorescence yield mode. The partial fluorescence yield (PFY) mode improves detection limits over total fluorescence or electron yield measurements when the concentration of the desired element in the sample is very low. The FY spectra of the concentrated samples can be distorted by self-absorption effect. This effect can be reduced either by diluting the concentration of the sample or decreasing the sample thickness (Ajiboye, 2008). The TEY detection mode is used to collect data for light elements with low fluorescence yield and the analysis is conducted under ultra-high vacuum conditions. Self-absorption effects are not observed in TEY spectra because the penetration depth of TEY measurement is higher than the escape depth. The escape depth of the electrons for TEY detection method is < 10 nm (Frazer et al., 2003), whereas FY method has an estimated depth of 70-100 nm (Katsikini et al., 1997). Thus, TEY is a surface sensitive technique, whereas FY probes more of the bulk material.

2.7 Summary

In this review the forms and chemistry of P in different soils, along with various approaches to assessing P forms have been discussed. It has been shown that the availability and transformation of applied P as mineral fertilizers and organic amendments depends on their chemical form and soil conditions such as pH. Characterization of the different forms of P

present in soil and an understanding of their behavior is essential to predict the fate of soil applied inorganic or organic P amendments. Very little work has been done on molecular level identification of P in prairie soils. The combined use of wet chemical analysis and molecular scale spectroscopic analysis as described in the following chapters should provide valuable new information on specific P forms and their chemistry in manure and fertilizer amended prairie soils.

CHAPTER 3.0

EFFECTS OF PLANT GROWTH AND TIME ON PHOSPHORUS SPECIATION IN A MANURE-AMENDED PRAIRIE SOIL UNDER CONTROLLED CONDITIONS

3.1 Preface

In the Canadian prairies, livestock manure can be an important source of P for crop production. The fate of applied manure P depends upon the chemical forms in which the P is contained or produced once in the soil. This is related in part to the type of manure and the presence or absence of growing plants in the soil that has received the manure. To assess how bioavailability and transformation of applied manure P is impacted by different sources of manure (liquid hog versus solid cattle) and the presence or absence of plants (canola) a growth chamber study was performed to follow P forms over a very short time period following application. Speciation in prairie soils receiving liquid hog or solid cattle manure was followed over a period of one to five weeks, with and without canola plants growing on the soils. A sequential chemical extraction and XANES spectroscopy were used to assess effects on soil P speciation.

3.2 Introduction

Livestock manure has long been recognized as an important source of nitrogen (N) for crop production in North America (Campbell et al., 1986; Chase et al., 1991). Meeting crop N requirement based on manure application often supplies P in excess of crop P requirements (Qian et al., 2004) which can accelerate the risk of P export off site and eutrophication in surface water (Turner and Leytem, 2004). Determining the distribution of P among forms and transformations over time in soil is essential to develop best management practices that will minimize adverse environmental impacts of manure application to agricultural soils. The P distribution among

forms varies among animal manure types due to variable P concentration and chemical composition in animal feeds, manure storage methods and stage of decomposition prior to application (Sharpley and Moyer, 2000; Toor et al., 2005; Negassa and Leinweber, 2009). Kashem et al. (2004) reported that greater amounts of labile P fractions were extracted from soils amended with cattle manure compared to hog manure applications, but the labile P fraction increased with the manure application rates from both types of manure. Many researchers (Hedley et al., 1982; Wagar et al., 1986; Qian and Schoenau, 2000; Kashem et al., 2004) also have performed short-term (1 to 16 weeks) incubation studies to examine manuring effects on the distribution of soil P among different forms. They have reported that most of the P from manure sources is recovered as inorganic labile P. Qian and Schoenau (2000) performed a growth chamber study and reported that supply rates of available P in the soil were significantly higher in the swine manure applications than in either unfertilized or urea-applied soils. It is known that liquid hog manure and solid cattle manure differ in P content and organic matter content, with liquid hog manure typically being lower in both P and organic matter content. Thus, the measurement of P forms in soil derived from different manure sources, and changes in these forms with time in the presence and absence of plant growth can provide insight on how these amendments affect P bioavailability and mobility in soil over the very short-term.

The fate of manure P in soils depends on the bioavailability of the P species initially present and the soil chemical properties such as pH, Ca levels, and mineral constituents that affect the kinds of P compounds formed in the soil over time (Tiessen et al., 1984; Vadas et al., 2007). Vadas et al. (2007) reported that the fate of P in dairy and poultry manure that was applied in field experiments and tracked over 14 months in Texas and Pennsylvania, USA was a rapid decrease in water extractable P for the first two months and then other forms of manure P

appeared to be gradually transformed to water extractable forms over time. However, there has been little work that has examined the effect of manure addition on P forms that arise in the very short term (weeks to months) in calcareous prairie soils typical of the northern prairies of North America. Depending on manure type, initial forms may be more reactive and dynamic compared to P reaction products formed over longer time scales. Furthermore, the presence of plant growth and rhizosphere activity may influence both the speciation and fate of manure P in the weeks following application. Understanding the distribution of various P forms in soils receiving different types of manure immediately following application may thus help in developing practices that will maximize crop utilization and minimize potential P loss.

In this experiment, a sequential chemical P fractionation procedure was used to measure the available and stable P forms as affected by time, plant growth and different sources of manures (cattle and hog manure). The sequential chemical analysis procedure has been widely used to determine P solubility in different manure and biosolid-applied soils (Kumar et al., 1993; Mozaffari and Sims, 1996; Gale et al., 2000; Qian and Schoenau, 2000; Adeli et al., 2005; Lehmann et al., 2005; Kar et al., 2011), and it has also been proven suitable to determine relatively small changes in soil P in short-term experiments (Hedley et al., 1982; Buehler et al., 2002). This procedure provides a rapid estimate of operationally defined pools of P (e.g. bioavailable P, labile and/or moderately labile, stable inorganic and organic P, and more stable residual P). These operationally defined fractions are based upon P extractability with extractants such as water and ion exchange resin, $NaHCO_3$, NaOH, HCl, and H_2SO_4 extractants (Sharpley and Moyer, 2000; Seiter et al., 2008), respectively.

Molecular-scale spectroscopic techniques have been increasingly utilized to accurately identify the chemical species of P in soils. Whereas sequential extraction techniques can separate

different P fractions based on solubility in different chemicals (Chang and Jackson, 1957; Hedley et al., 1982), they cannot provide any direct structural information about the solid state chemistry of P in manure and manure-amended soils (Turner and Leytem, 2004; Shober et al., 2006). Synchrotron-based P K-edge XANES spectroscopy has been widely used to accurately identify P minerals present in manures (Peak et al., 2002; Toor et al., 2005), and whole soils (Hesterberg et al., 1999; Beauchemin et al., 2003; Shober et al., 2006; Ajiboye et al., 2008; Kruse and Leinweber, 2008; Kar et al., 2011; Peak et al., 2012).

Phosphorus K-edge XANES spectroscopy is both element specific and nondestructive (Beauchemin et al., 2003) and allows analysis of samples without additional sample treatment (Kruse and Leinweber, 2008). This technique is able to characterize poorly crystalline, amorphous materials and sorption species by comparing the spectra of representative standards with the spectra of unknown samples (Shober et al., 2006). The general approach used by several researchers (Beauchemin et al., 2003; Toor et al., 2005; Kruse and Leinweber, 2008; Seiter et al., 2008) is to combine P XANES spectroscopy (to directly probe solid state P speciation) with sequential chemical extraction (to estimate the solubility and lability of soil P) on the same samples. However, while many studies of P speciation have been completed, there remains a need to understand the changes in P pools as related to plant growth. In this study, we characterize the P species in soil amended with two types of manures in the presence and absence of plants (canola) growth over a period of one to five weeks under controlled conditions.

3.3 Materials and Methods

3.3.1 Soil and manure sources

Soil used in this study was collected from approximately 6.5 km west of the town of Humboldt, Saskatchewan, Canada at a long-term manure field trial site (Schoenau et al., 1999).

The soil type at the study area is from a midslope position of an Orthic Black Chernozem of the Cudworth Association which is developed from loam textured, calcareous, silty-lacustrine deposits and having very gently sloping (0-2%) topography (Saskatchewan Soil Survey, 1989). The soil was collected from the surface layer (0 to 15 cm) of an unfertilized (no manure, no commercial fertilizer for last ten years) control area. The larger undecomposed plant material was removed from the collected soil and the remaining soil was then air-dried, crushed, passed through a 2-mm sieve, and stored at room temperature. Selected soil characteristics were measured as follows: Soil texture was determined using a HORIBA LA-950 Laser diffraction particle-size analyzer (HORIBA Scientific Inc., Edison, NJ, USA). Soil pH and electrical conductivity (EC) were measured with a 1:2 soil/water suspension using a glass electrode (Peters et al., 2003). Organic carbon (OC) was determined using the dry combustion method using a LECO-C632 carbon analyzer (LECO© Corporation, 1987). Available P was determined by a modified Kelowna method (Qian et al., 1994). Results from these selected soil analyses are summarized in Table 3.1.

Table 3.1. Some basic characteristics of the soil used for the growth chamber study.

Soil Association	Texture	pH	EC†	OC‡	TC ¶	Available P*
			(mS cm^{-1})	—— % ——		(mg kg^{-1})
Black Chernozem	Loam	8.1	0.71	3.13	4.37	20.3

†EC, electrical conductivity; ‡OC, organic carbon; ¶TC, total carbon, *Available P was measured by modified Kelowna method

Solid cattle manure (SCM) used in the study was collected from the Poundmaker Feedlot, which is located approximately 8 km east of the town of Lanigan, Saskatchewan. The SCM was collected by sampling at various points of the feedlot stockpiles following the Tri-Provincial manure application and sampling guidelines (Saskatchewan Agriculture and Food, 2003). The liquid hog manure (LHM) was collected from a single cell earthen storage unit at

Possberg Pork Farms (approximately 6 km south of the Dixon site). Both types of manure collected were immediately frozen and stored in 10 L plastic containers at -20 °C. Before use, containers were removed from frozen storage and thawed to room temperature. After thawing, sample containers were opened in the laboratory fume hood and stirred to homogenize the contents before application. Immediately before application, a 5.0 g manure sample was taken and analyzed as follows: Total P content was determined using a standard H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967), followed by determination of concentration in the digest colorimetrically (Murphy and Riley, 1962). Available N (ammonium-N and nitrate-N) was determined colorimetrically by KCl extraction (Keeney and Nelson, 1982) and nitrate-N was below detection limits. Available P in the solid and liquid manure was measured colorimetrically after adsorption via an anion exchange resin membrane sorption technique (Qian et al., 1992). The contents of N and P in manure samples are shown in Table 3.2.

Table 3.2. Phosphorus and nitrogen contents of solid cattle manure (SCM) and liquid hog manure (LHM) used in this study (fresh weight basis).

Manure	Total-P	Resin-P	Total-N	NH_4-N
	mg kg^{-1}			
SCM	3723	156	4948	402
LHM	207	5	2400	2100

3.3.2 Growth chamber experiment

The plant growth experiment was a pot study conducted under controlled environment (growth chamber) conditions. The experiment was carried out using two treatments (solid cattle manure and liquid hog manure) with two application rates. The low rates were 6 g and 16 mL 800 g^{-1} soil equivalent to 15 T ha^{-1} and 40 KL ha^{-1} for the SCM and LHM, respectively while the high rates were four time higher than these rates. These rates were selected to represent the range of rates typically applied in the field in Saskatchewan for these two manure types. The low rates

are amounts that would be applied in a single application intended for one year while the high rate represents a large single application intended to provide nutrient for three to four years (Table 3.3). The treatments are referred in this study as control, SCM low, SCM high, LHM low, and LHM high. A high rate was also desirable to increase the likelihood of being able to identify P species in the XANES technique. Each treatment was replicated three times. Eight-hundred grams of air-dried soil was mixed thoroughly with solid cattle and liquid hog manure. The same amount of water was added to the control soils to maintain soil moisture at the same level as all the other treatments. The soil was adjusted 50% of field capacity before seeding. Eight canola (*B. napus* var. *Invigor*) seeds were distributed over the surface of each pot and placed approximately 2 mm below the soil surface. The plants were watered every day with tap water to keep soil moisture near field capacity in all treatments. The pots were arranged in a completely randomized design and were rotated inside the growth chamber each week during the 5 week experimental period. Growth chamber conditions were maintained at a 16 hour day-length (at 25 ^{o}C) and 8 hour night-length (at 12 ^{o}C). Relative humidity was maintained approximately at 50% and light intensity was 45,600 LUX. Seedlings were thinned to three plants per pot after germination. Plants were grown for 5 weeks and then harvested. Soil samples were collected using a micro-coring device 1 week, 3 weeks and 5 weeks after seeding, and the resulting hole was plugged with the same soil used for this study. The collected soil samples were air-dried, ground, and analyzed for P forms by sequential chemical extraction (Tiessen and Moir, 1993) and P K-edge XANES spectroscopy. After harvesting, plant samples were dried at 50^{o}C, ground, total P was determined colorimetrically (Murphy and Riley, 1962) using a Technicon Autoanalyzer after acid-digestion analysis (Thomas et al., 1967).

3.3.3 Sequential fractionation scheme

The fractionation scheme used to separate the soil P into different fractions is described by Tiessen and Moir (1993). Briefly a 0.5 g sample of air-dried soil was first extracted with 30 mL of deionized water containing a HCO_3 saturated strip of anion exchange resin membrane. Then the residue of the resin extraction is extracted, and the process repeated every 16 h using progressively stronger extractants. The fractionation scheme utilized 30 mL of 0.5 *M* $NaHCO_3$ (pH 8.5) after water followed by 0.1 *M* NaOH, and 1.0 *M* HCl extractants. For resin extraction, the resin membrane was removed from soil suspension and shaken with 20 mL 0.5 *M* HCl for 16 h in rotary shaker. After each extraction, the soil and water was separated via centrifugation for 10 min at 8,000 x g at 0 ^{o}C, and the supernatant was then filtered into the 40 mL vials under suction using a 0.45-µm Millipore filters without disturbing the organic matter sediment. This extraction process was repeated for 16 h in a rotary shaker for each extraction. At the end, the remaining soil residue from all soil samples was digested using standard H_2O_2-H_2SO_4 digestion method (Thomas et al., 1967) followed by colorimetry (Murphy and Riley, 1962) to measure any residual P that remained.

Table 3.3. Rates of total and available P and N added as solid cattle manure (SCM) and liquid hog manure (LHM) at the low and high application rates.

Treatment	Total-P	Resin-P	Total-N	NH_4-N
	kg ha^{-1}			
Cattle manure				
Low	56	2.3	74	6
High	223	9.2	297	24
Hog manure				
Low	8	0.21	85	75
High	32	0.84	340	300

Phosphorus concentrations after each step of extraction were determined using an ascorbic acid colorimetric method (Murphy and Riley, 1962). Resin membrane extractable P and HCl extractable P were directly determined, but inorganic P (P_i) in $NaHCO_3$ and NaOH extracts was determined after precipitating the organic matter by acidifying the filtered samples with 5 mL 0.9 *M* H_2SO_4. Total P (P_t) in $NaHCO_3$, NaOH, and HCl extracts was determined after digesting the filtered samples with 0.5 g $(NH_4)_2S_2O_8$ and 10 mL of 0.9 *M* H_2SO_4. The organic P (P_o) in all of these extracts was calculated by the difference between P_t and P_i in each extract. A spectrophotometer (Beckman DU-65) was used to measure absorbance at wavelength of 712 nm. A more detailed description of this fractionation method can be found in Kar et al. (2011).

3.3.4 Phosphorus K-edge XANES spectroscopy analysis

Solid-state characterization of P from the soil samples, manure, and P standards was conducted at the Soft X-ray micro-characterization beamline (SXRMB) at the Canadian Light Source (CLS), Saskatoon, Canada. Measurements were performed with InSb(III) monochromator with focused beam size 300µm×300µm. Beamline was calibrated using $ZnPO_4$ powder to the edge energy of 2158 eV in total electron yield (TEY) mode spectrum. The P standards spectra were collected in the TEY mode to minimize self-absorption, which can distort fluorescence measurements of concentrated samples and standards (Toor et al., 2005; Kar et al., 2011, Peak et al., 2012). P XANES spectra were collected from a small amount of air-dried soil sample removed from SCM and LHM high rate pot study treatments and analyzed for differences in P speciation at three different times, using a difference spectra approach. For comparison, air-dried manure samples and a variety of organic and inorganic phosphate standards were collected. The samples were mounted on a stainless steel sample holder using double-sided carbon tape and placed in the vacuum chamber. Soil and manure sample spectra

were collected in partial fluorescence yield (PFY) mode from 2110-2190 eV with a step size of 1 eV on pre-edge region (2110-2145 eV), 0.25 eV in near-edge region (2145.25-2180 eV), and 0.5 eV in post-edge region (2180.5-2200 eV) with a constant dwell time of 4.0 s. Multiple co-added spectra were averaged to obtain adequate signal to noise ratio for analysis. XANES spectra were analyzed using WinXAS 3.1. software. The averaged XANES spectra were background corrected by a first-order polynomial fit through the pre-edge region (2135-2145 eV). This was followed by normalization to an edge jump of 1.0 between 2140 and 2180 eV to facilitate comparison with samples of different P concentration.

3.3.5 Statistical analysis

The fractionation data in each treatment at the presence and absence of plants were analyzed by student's *t*-test using Statistical package of Social Science (SPSS Inc.) at $P \leq 0.05$ probability level. The significance of treatments and time effects as well as interaction of treatments and time effects was analyzed using one way analysis of variance (ANOVA) using Statistical Analysis Software (SAS Institute, Inc. 2011) of fixed effects model and determined at $P \leq 0.05$ probability levels. Multiple comparisons of time effect were performed using the Tukey's test. In all cases, the results are presented as means of triplicates.

3.4 Results and Discussion

3.4.1 Effects of plant growth on soil phosphorus forms

The wet chemical P fractionation results revealed a significant effect of the presence of canola plants on soil P forms. In general, the presence of plants was observed to reduce P concentration in most P fractions, with the exception of LHM amended soil, in which plants had no significant effect (Figure 3.1).

The resin extractable P was numerically lower in all treatments with plants present. The decrease in the LHM treatments (both low and high) and in the SCM low rate was not statistically significant, however (Figure 3.1).

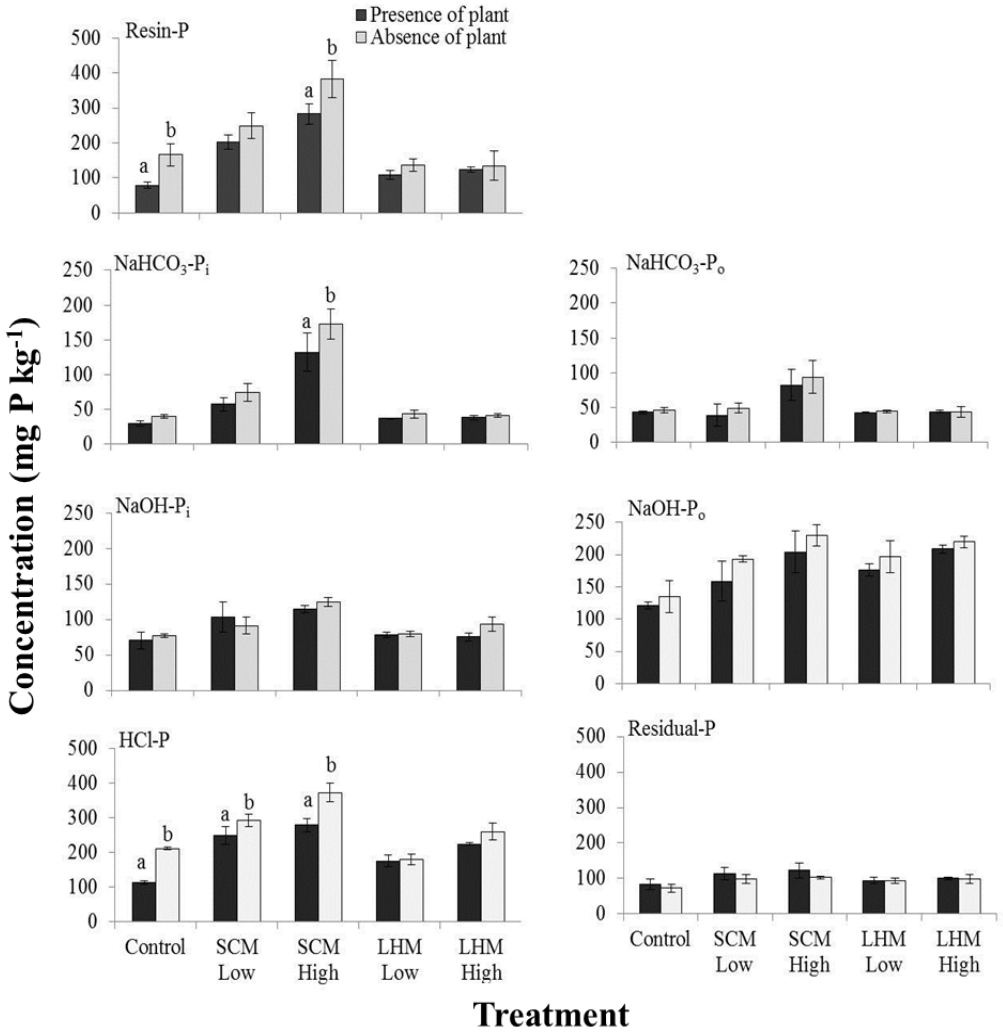

Figure 3.1. Distribution of sequentially extracted inorganic (P_i) and organic P (P_o) fractions in SCM and LHM applied soils after five weeks in the presence or absence of plant growth. Organic P was calculated as P_t-P_i. Low and High refers to solid cattle manure applied @ 15 and 60 T ha^{-1}, respectively and LHM low and high refers to liquid hog manure @ 40 and 160 KL ha^{-1}, respectively. Error bars represent standard deviation (n = 3). Columns in each treatment followed by a different letter are significantly different ($P \leq 0.05$).

For the sequential extractions, the same trend was observed in $NaHCO_3$-P_i and P_o fractions; the amount of the $NaHCO_3$-P_i fraction in all treatments was decreased in presence of plants but the

only significant difference was observed in the high rate of SCM application (Figure 3.1). The amount of labile P_i (resin-P + $NaHCO_3$-P_i) was much higher in solid cattle manure amended soil (426 and 260 mg P kg^{-1} soil at high and low rates, respectively) than liquid hog manure amended soil (163 and 145 mg P kg^{-1} soil at high and low rate, respectively). The amount of labile P_i (resin-P + $NaHCO_3$-P_i) was much higher in solid cattle manure amended soil than liquid hog manure amended soil reflecting that the amounts of manure P added were much higher in the SCM treatments than the LHM treatments (Table 3.2 and Table 3.3). Qian and Schoenau (2000) conducted a short-term experiment in a Black Chernozemic soil in Saskatchewan and reported that a single application of swine manure at low and high rates did not have a significant impact on increasing labile soil P because of lower amounts of available and total P in swine manure compared to cattle manure.

Plants had less effect on concentrations of the moderately labile (NaOH-P) forms compared to the labile forms (resin-P + $NaHCO_3$-P); there was no significant decrease or increase in any fraction for all treatments (Figure 3.1). Rhizosphere acidification by the plants is anticipated to have more effect on $NaHCO_3$-P_i fraction as observed, and would have led to less depletion of NaOH-P_i fraction since NaOH is believed to extract mainly Al and Fe associated P. It can also be inferred that the P_o fractions were not a significant contributor to soil available P, perhaps because labile P_i was high (Guo et al., 2000) and that may inhibit enzymatic hydrolysis of P_o ester phosphate bonds. Beck and Sanchez (1994) conducted a repeated P application experiment in an Ultisol soil and reported that P_o did not significantly contribute to plant-available P_i when large amounts of P_i were the major P source for plant growth. Although the soil properties are quite different, the same trend is observed in our Chernozemic soil.

The highest amount of HCl-P was observed in the SCM high rate application treatment in both the presence and absence of plants (Figure 3.1). Both the low and high rates of SCM and the control soil showed significantly lower P content in the presence of plants (Figure 3.1), but LHM did not show any significant plant-induced depletion despite high yields (Figure 3.2). The decrease of the more stable P form (HCl-P) in the presence of plants could be due to transformation of this fraction into more labile forms by rhizosphere acidification (Bertrand et al., 1999). Grinsted et al. (1982) reported that rhizosphere acidification increased P availability and uptake due to the dissolution of inorganic Ca-P minerals in soil. Plants also release H^+ ions to balance charges caused by an excess of cation absorption in relation to anion absorption (Hedley et al., 1982; Moorby et al., 1988). The significant decrease of labile P_i in SCM amended and control soil could cause enhanced depletion of HCl-P to replenish that labile P_i fraction. This seems to be the best explanation for our results, although we do not have direct confirmation of a pH decrease occurring in this study. Residual P did not follow the same trend as the other fractions and was relatively unaffected by plant growth. This suggests that the canola plants had very little access to the residual fraction, and is consistent with the opinion that the residual fraction is a stable fraction that has little impact on plant available P in the short term.

3.4.2 Changes in soil phosphorus forms with time

Time and treatment had a significant effect on the P fractions (Table 3.4). To illustrate the changes in P speciation over time, the control soil and high rates of SCM and LHM were compared after 1, 3, and 5 weeks of addition in the presence of growing canola plants (Figure 3.3 and Appendix A).

Manure addition resulted in a significant increase in resin P with time. In terms of net change, the LHM amended soil had a larger increase in resin P (100 mg P kg^{-1} soil) from week 1

to week 5 than did the SCM amended soil (23 mg P kg^{-1} soil) (Figure 3.3 and Appendix A), even though the amount of P added as manure to the LHM soil was less than in the SCM soil.

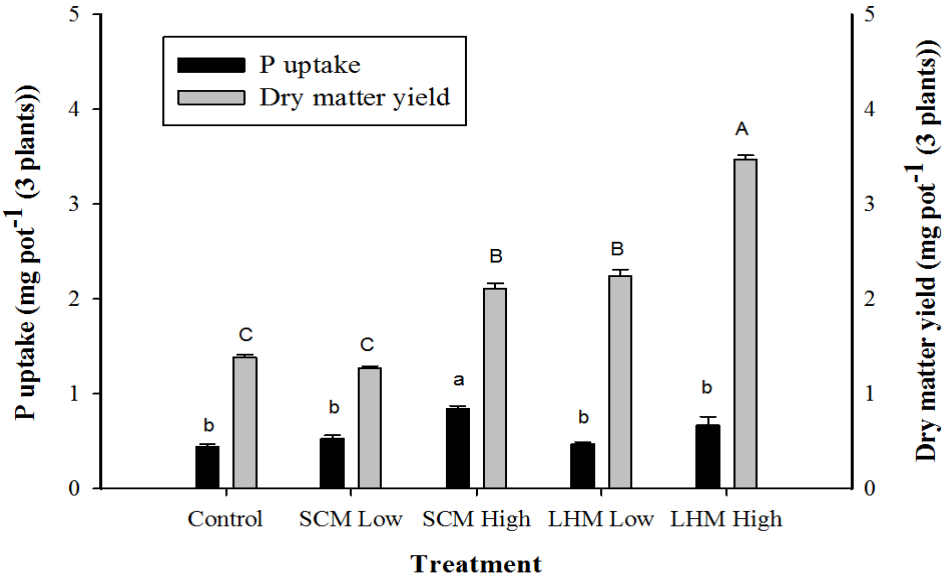

Figure 3.2. Phosphorus uptake (black bars) and dry matter yield (gray bars) by canola after 5 weeks growth in controlled environment. Error bars represent standard deviation (n = 3). For P uptake, values in columns followed by different lowercase letters indicate significant treatment effects ($P \leq 0.05$); and for dry matter yield, values in a column followed by different uppercase letters indicate significant treatment effects ($P \leq 0.05$).

The larger amount of available nitrogen added to the LHM soil (Table 3.3) and the associated stimulation of plant and microbial growth in the first weeks may have mobilized relatively more P from stable forms compared to the SCM amended soil.

The amount of labile $NaHCO_3$-P_i was increased in both the unamended and manure amended soils. The SCM amended soil showed significant net changes with time as the net increase was 78 mg P kg^{-1} soil over week 1 to 5 (figure 3.3 and Appendix A) but LHM amended soil did not significantly increase in $NaHCO_3$-P_i content with time. The labile $NaHCO_3$-P_o increased or decreased depending upon manure type and the unamended soil also had a small increase of $NaHCO_3$-P_o.

Table 3.4. Analysis of variance (ANOVA) of the effect of treatments (high rate) and time (1, 3, and 5 week) on the different P forms at presence of plants.

Factor	Resin-P	$NaHCO_3$-P_i	$NaHCO_3$-P_o	NaOH-P_i	NaOH-P_o	HCl-P	Residual-P
Treatment (df=2)							
P value	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001	0.0114
SEM¶	6.56	4.49	4	1.93	4.74	3.88	5.21
Time (df=2)							
P value	<.0001	<.0001	0.0021	<.0001	<.0001	<.0001	0.1825
SEM¶	6.56	4.49	4	1.93	4.74	3.88	5.21
Treatment x Time (df=4)							
P value	0.0177	<.0001	0.0596	0.0007	0.0009	<.0001	0.4688
SEM¶	10.85	6.31	6.93	3.34	8.21	6.72	9.02

¶SEM, standard error of mean

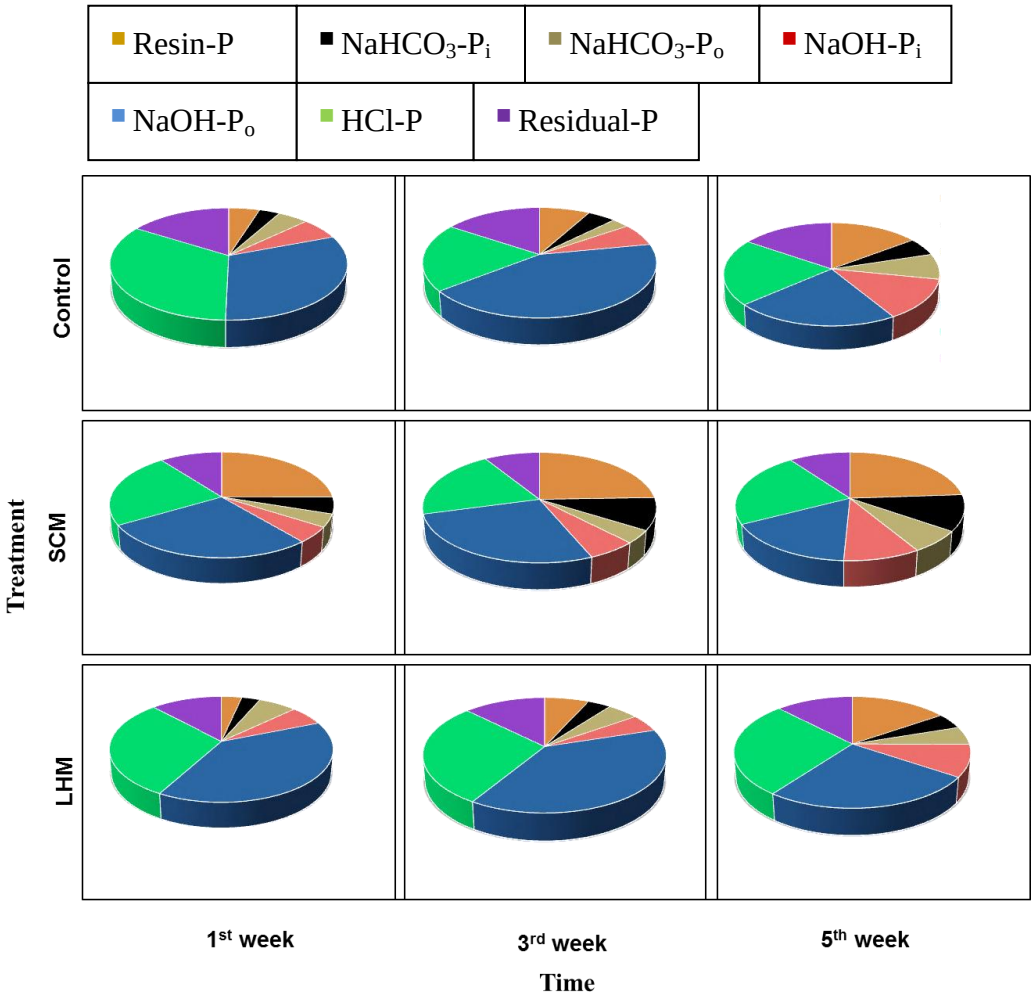

Figure 3.3. Distribution of the different P forms for the unamended control, SCM, and LHM amended soil (high rate) at 1, 3, and 5 weeks after addition in the presence of plants.

The highest increase in P_o fraction was observed in SCM, whereas a decrease was observed in LHM amended soil, but the magnitude of net changes was very small compared to the $NaHCO_3$-P_i fraction.

The largest proportion of P was extracted as moderately labile P (NaOH extractable P_i and P_o) in both amended and unamended soils (Figure 3.3 and Appendix A). The organic fractions of NaOH extractable P were much higher than inorganic fractions for all soils. Compared to control, both manure types increased the NaOH-P_i fraction, but SCM showed the net highest increase (62 mg P kg^{-1}) with time after 5 weeks of plant growth. The amount of

$NaOH\text{-}P_o$ fractions decreased with time in both manure amended and unamended control soil. The increase of resin-P, $NaHCO_3\text{-}P_i$, and $NaOH\text{-}P_i$ in manured soil from week one to week five probably reflects a net conversion of stable organic P forms such as $NaOH\text{-}P_o$ and stable inorganic P to inorganic forms in all soils with time (Kashem et al., 2004) perhaps associated with rhizosphere activity such as phosphatase enzymes and acidification (Armstrong and Helyar, 1992). The resin-P is usually considered as the readily plant available form (Amer et al., 1955; Wager et al., 1986) which originated from slightly soluble calcium phosphates and loosely adsorbed P_i on the surfaces of metal oxides or carbonates (Mattingly, 1975). The significant increase of $NaHCO_3\text{-}P_i$ and P_o with time in SCM amended soil also implies that the some portion of most labile fractions (resin-P) could also be transformed to less labile fraction ($NaHCO_3$-P) as a result of sorption processes (Kashem et al., 2004).

The second largest P fraction was the P fraction removed by HCl in our fractionation scheme (Figure 3.3). The highest depletion of HCl-P (94 mg P kg-1 soil) was observed with time (after both 3 and 5 weeks) in unamended control soil whereas no net changes were observed in any manure amended soil. Residual P increased slightly in manure amended soils and decreased in unamended control soil, but the changes of this fraction over time were very small in all cases. The gradual decrease of Ca bound P and gradual increase of resin-P in unamended control soil suggest a transformation of soil HCl-P to more readily available P forms with time (Guo et al., 2000). The slight increases of residual P over time with manure addition could be due to some recalcitrant forms of P forming from decomposition (Iyamuremye et al., 1996). The patterns of P change in each fraction were very similar in the presence or absence of plant growth, but the labile P fraction (resin-P + NaHCO3-Pi) was much lower in presence of plants after 5 weeks,

reflective of a large uptake of labile P by the canola plants, especially in the soil amended with SCM (Figures 3.2 and 3.3).

3.5 XANES analysis

The background-subtracted normalized XANES spectra of the unamended control soil, solid cattle and liquid hog manure amendments, and dicalcium phosphate (brushite) are shown in Figure 3.4. The manure samples weakly exhibit some spectral features generally consistent with calcium phosphates which are consistent with the typical speciation of animal manures (Sato et al., 2005; Toor et al., 2005). The position of these peaks changes in LHM amended soil, which may be evidence for a slightly different crystallinity or structure of $CaPO_4$ species present upon LHM addition. All of the distinctive Ca-P features in the XANES spectra of both manures disappear after they are mixed with the soil. Other than the intensity of the absorption edge (due to differences in P concentration) in non-normalized data (not shown), no obvious differences in the spectra of the amended soils can be observed. This shows that the manure P is redistributing into the soil over the course of this pot study. For this reason, we used the approach of taking difference spectra (subtracting one sample spectrum from another to probe the species that is lost or changed for more detailed analysis. Additional details of this differential XANES spectral analysis can be found in Kar et al. (2011).

XANES spectra for SCM amended soil in the presence and absence of canola plants were compared (Figure 3.5) after 5 weeks of growth to examine the effect that plants had upon P speciation. The spectra in LHM amended soil are not included in Figure 3.5 because we did not see any significant effect of plants in LHM amended soil (Figure 3.1). The spectrum of P removed by the plant can be obtained by subtracting the background-subtracted spectra of soil with plants after 5 weeks from the initial plant-free soil.

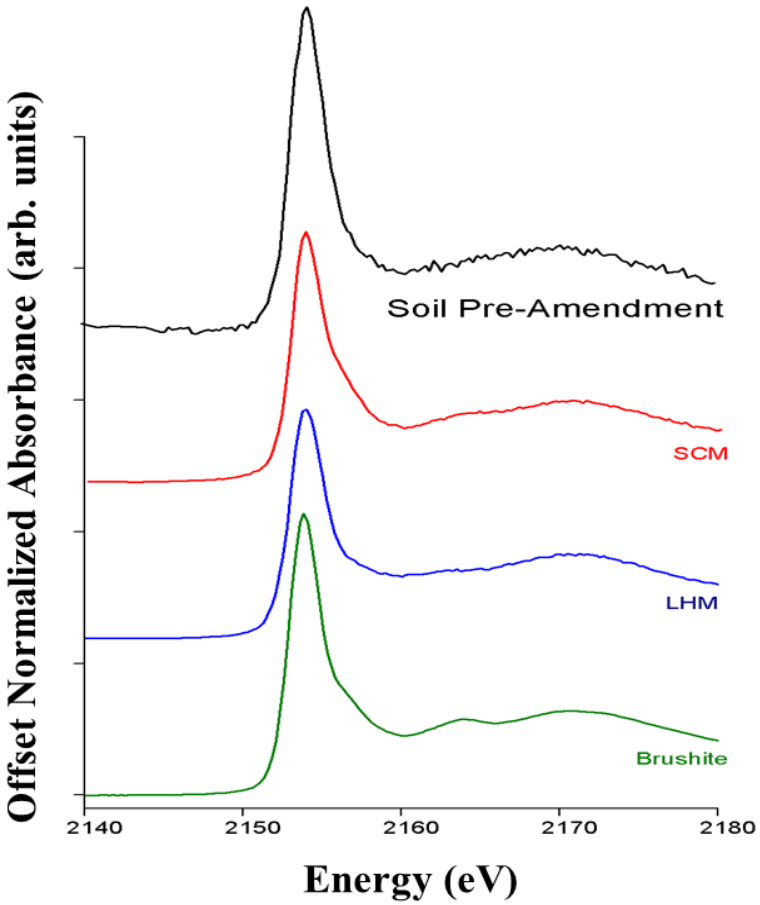

Figure 3.4. Normalized P K-edge XANES spectra of control soil (soil pre-amendment), solid cattle manure (SCM), liquid hog manure (LHM), and dicalcium phosphate (brushite).

To facilitate comparison, after obtaining the differences, all spectra were normalized to a maximum absorbance of 1.0. No changes in the structure of this difference spectrum from the original samples can be observed; the normalized difference has no structure. This implies that either the plants access all of the P pools in roughly equal proportions so that none of the fractions are changing or that manure is contributing to replenishment of the fractions relatively equally.

The XANES data were also analyzed to see the combined effect of plant growth and time on P speciation (Figure 3.6). For this comparison, the difference spectra were produced by subtracting the background-subtracted spectrum of samples after 5 weeks of plant growth from a

sample after 1 week with no plants; this was done for both SCM and LHM amended experiments.

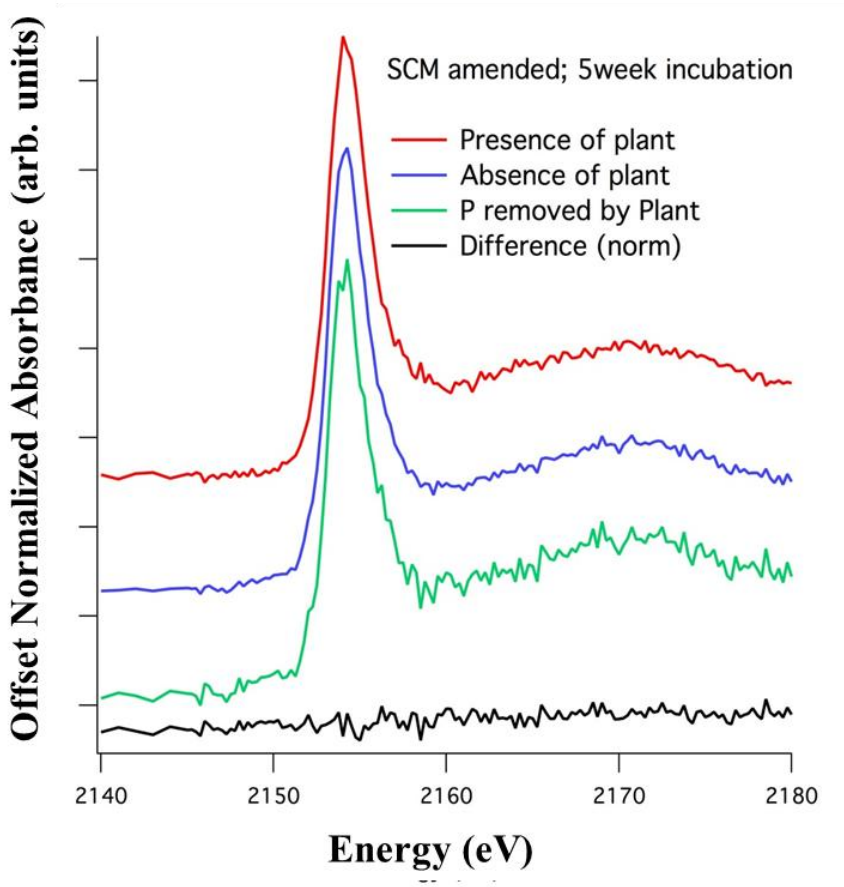

Figure 3.5. Comparison of the spectra of SCM amended soils in the presence or absence of plant growth. The spectrum of P removed by plants is obtained by subtraction of spectrum from soil after 5 weeks plant growth from 5 weeks without plants. All samples are normalized to the same maximum intensity. No changes in P speciation can be observed.

For the SCM amended soil, we did not see any detectable speciation change with time or plants. In contrast, there is a small speciation change in the LHM amended soil as the main P peak shifted to slightly higher energy in the difference spectrum (arrow in Figure 3.6b). In general, the observed changes in speciation are far smaller than have been observed when performing sequential chemical extractions on soils (Kar et al., 2011), or even upon desorbing a soil with a weak desorbing agent (Peak et al., 2012).

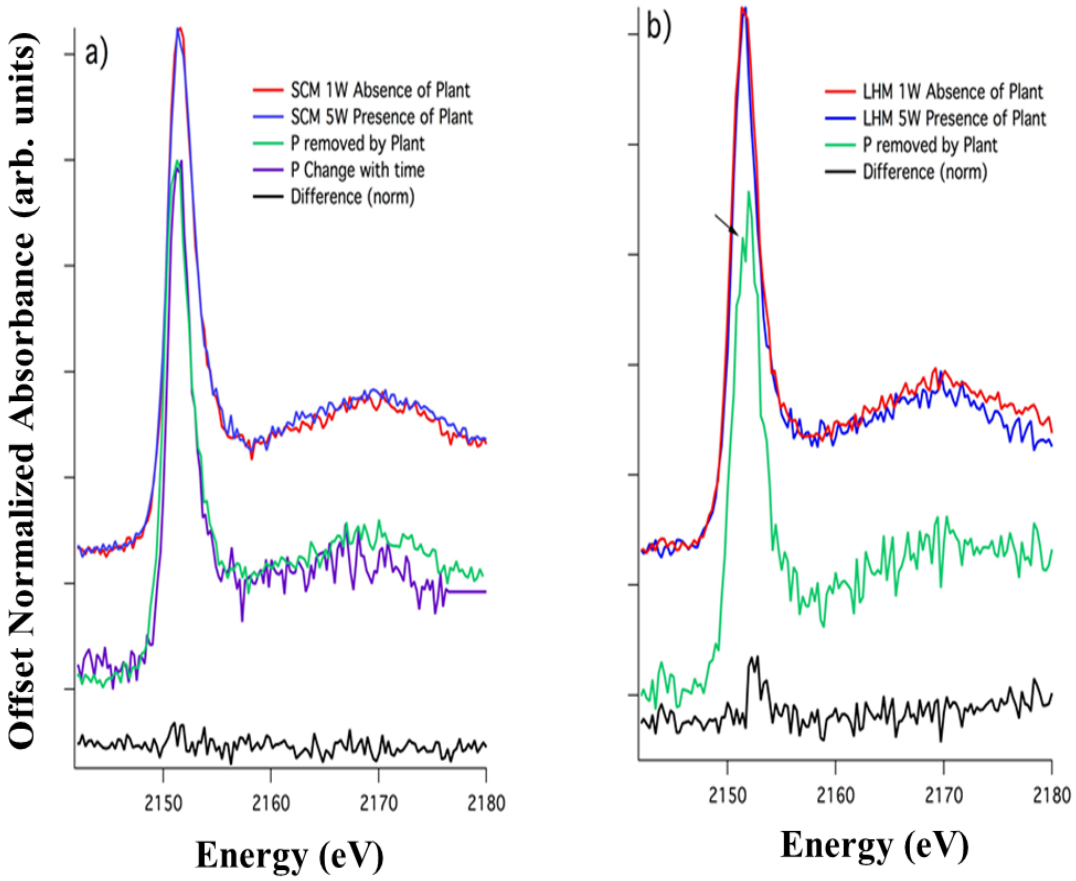

Figure 3.6. Comparison of the effect of time in SCM (A) and LHM (B) amended soil 5 weeks after manure addition in the presence or absence of plant growth. Desorption spectrum of P removed by plants is obtained by subtraction of spectrum of 5 weeks plant growth from first week without plant growth.

The major shift in speciation appears to occur rapidly upon the initial addition of manure to the soils; subsequent changes in speciation as a function of plant growth or time are not measurable with XANES. One reason for this could be that many of the labile P fractions are not easily distinguishable using P K-edge XANES. For example, P K-XANES spectra of a variety of phosphate standard compounds are shown in Figure 3.7.

It is not always possible to distinguish adsorbed, organic, poorly crystalline calcium phosphates (i.e. monetite), and aqueous phosphate from one another. In a soil, several of these fractions may occur simultaneously, and redistribution among adsorbed, aqueous, organic, and

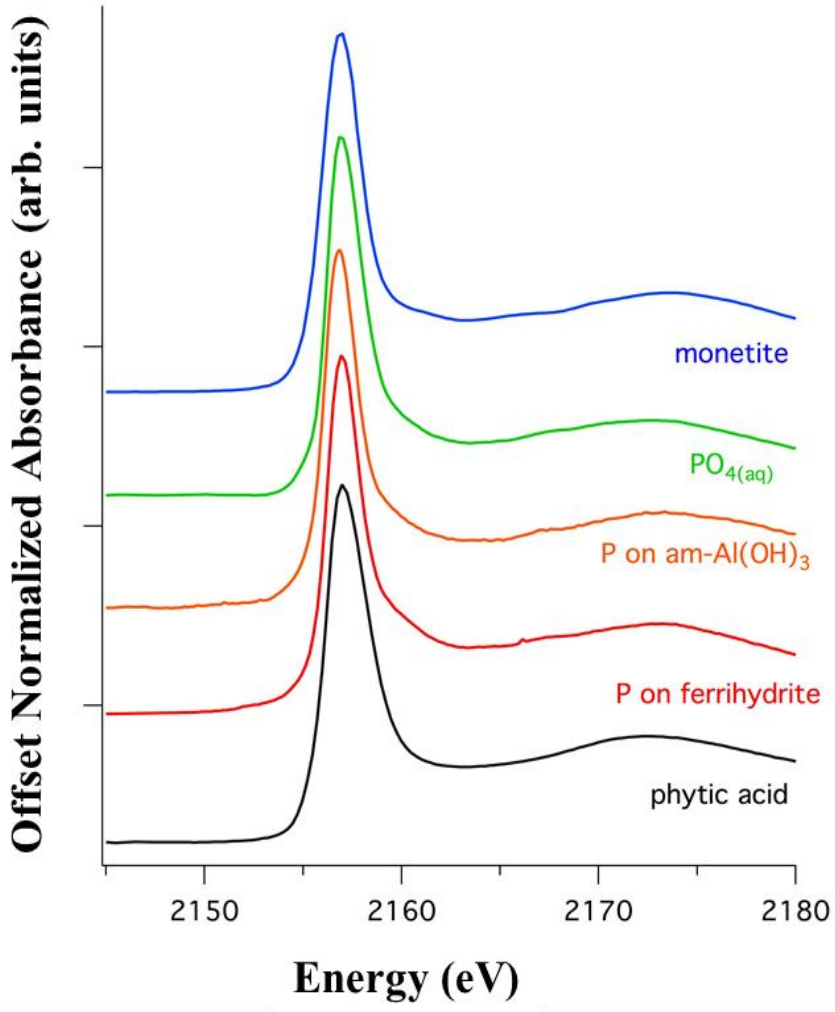

Figure 3.7. Normalized and background subtracted P K-edge XANES spectra of phosphate standards of dicalcium phosphates (monetite phase), aqueous phosphate, phosphates adsorbed on an Al and Fe oxides, and organic phosphate (phytic acid). Spectra are offset by 1 absorbance unit to allow clearer comparison of spectral features among the standards.

poorly crystalline Ca-P may not be detected by XANES spectroscopy. It does seem clear from our XANES measurements that no large changes in the bulk speciation or crystallinity of P are

occurring. It also appears that the initial redistribution of P upon mixing manure into a soil is the dominant chemical effect in this system.

3.5 Conclusions

The presence of plants decreased the concentration of the different P fractions in manured soil. This effect was most pronounced in soil amended with cattle manure and in the unamended control. The soil P fractions were least affected by plant growth in the soil amended with liquid hog manure. The resin-P, $NaHCO_3$-P_i, and HCl-P fractions in unamended control and SCM amended soil showed significant decrease after 5 weeks of plant growth compared to the treatments where plants were absent. The increase of resin-P, $NaHCO_3$-P_i and P_o, and NaOH-P_i observed over 5 weeks in all treatments indicates a replenishment of these fractions likely through mineralization of NaOH-P_o or solubilization of HCl-P. The XANEs results showed no large changes in the bulk speciation or crystallinity of P occured over 5 weeks in this pot study. Plants are generally effective in reducing concentrations of labile P fraction in the short term compared to conditions of no plant growth i.e. fallow.

CHAPTER 4.0

SPATIAL DISTRIBUTION AND CHEMICAL SPECIATION OF SOIL PHOSPHORUS IN A BAND APPLICATION

4.1 Preface

In the previous chapter it was shown that speciation changes among poorly crystalline, weakly bound adsorbed and organic P forms in manure amended soils over a period of five weeks were limited and were mainly related to plant growth and uptake. This chapter addresses the fate and transformation of applied P when applied as a band under field conditions assessed a few months following application. There are various factors governing the fate and transformation of applied P in the field including landscape position, as it affects pH, and soil constituents that can bind P such as Ca, Al, and Fe-P. Furthermore, there is limited information on how manure and inorganic fertilizer application in a concentrated band impacts the solubility, mobility, and transformation of the phosphate compounds in calcareous prairie soils under field conditions. This chapter examines the effect of these factors on spatial distribution and speciation of P in manure and inorganic fertilized Black and Brown Chernozemic soils, respectively. This chapter is published in the Soil Science Society of America Journal (Kar et al., 2012).

4.2 Introduction

Subsurface band application of P fertilizers is generally considered agronomically superior to broadcast or broadcast-incorporated applications for annual crops (Mulkey et al., 1979; Sleight et al., 1984; Sander et al., 1990; Sanchez et al., 1991; Koenig et al., 2009). Subsurface band application of P fertilizer may reduce soil-fertilizer contact and subsequent sorption or precipitation reactions (Prummel, 1957) and enhance the availability and solubility of P compared with broadcast applications in soil environments, especially where the soil surface is

subject to drying (Howard et al., 2002; Singh et al., 2005). Phosphorus movement is limited in soil from the injection zone after band application, although this depends on soil properties and other constituents present in soil (Eghball et al., 1990). Several researchers have estimated that P movement is 3 to 5 cm from the point of application as band injected granules over periods of 1 to 3 months (Heslep and Black, 1954; Lawton and Vomocil, 1954; Blanchar and Caldwell, 1966; Khasawneh et al., 1974; Eghball and Sander, 1989; Eghball et al., 1990).

The slope position in the landscape is also important in affecting the availability of labile P in farm fields. Some researchers (Wagar et al., 1986; Heilmann et al., 2005) have reported that the absolute amount and the proportion of different P fractions can be affected by slope position, even with identical management practices. Heilmann et al. (2005) conducted a study and reported that both labile and moderately labile P concentrations from fertilizer P were higher in the lower slope position than the upper slope position. Schoenau et al. (1989) also reported that labile and moderately labile P concentrations were higher in the downslope position than the upper slope position in Saskatchewan prairie soils. Therefore, different fertilizer management practices, and slope position in the field can affect the distribution and chemistry of manure and fertilizer P upon reaction with the soil when it is placed in bands.

Knowledge of P chemistry in the soil following fertilizer application is important in understanding the bioavailability and mobility of P in the environment (Ajiboye et al., 2008). Phosphorus availability depends on the P compounds that are present in inorganic fertilizer and manure, and that are formed in soil after application. Brushite, aluminum (Al), and iron (Fe) phosphate were observed after organic fertilizer (biosolids) application; while adsorbed and crystalline apatite type calcium phosphates were observed after long-term inorganic fertilizer applications in an Illinois soil (Kar et al., 2011). Animal manure contains various types of

calcium phosphates (Ca-P) and high concentrations of Ca-P in soils can have a profound effect on P solubility and availability (Penn and Bryant, 2008). The calcium carbonate ($CaCO_3$) content of the soil itself has a direct effect on P availability (Bertrand et al., 2003). Phosphorus availability is controlled by surface reactions between P and carbonates, precipitation reactions of P with Ca^{+2} as secondary Ca-P minerals in calcareous soils, and surface reactions with Fe and Al oxides and clay minerals, and precipitation as secondary Fe and Al-P minerals in acidic soils (Tunesi et al., 1999). Higher Ca:P ratio was reported to have favoured precipitation reaction while lower Ca:P ratio favoured precipitation and adsorption reactions when soil was amended with inorganic fertilizer (mono-ammonium phosphate) in a calcareous soil (Ajiboye et al., 2008).

The transformation of P applied to soil in a band is postulated to be affected by P form (manure versus fertilizer) because of differences in P chemical form, solubility (Kashem et al., 2004) and the ability of organic ligands present in manure to block P adsorption sites. Manure may also change P availability by altering soil microbial activity (Barrow, 1960). Changes in chemical form with aging can result in less fertilizer P being available for plant uptake over time (Laboski and Lamb, 2003). However, it is unknown how inorganic fertilizer or manure application in bands impacts the solubility and transformation of the phosphate compounds in prairie soils directly in the center of the band and extending outwards. The characterization of the chemical species of soil P in the band and adjacent regions should help to better understand the fate of added P in these systems.

X-ray absorption near-edge structure (XANES) spectroscopy is a molecular-scale analytical technique that has been proven to decisively identify P species present in animal manure (Peak et al., 2002; Toor et al., 2005), and whole soils (Hesterberg et al., 1999; Beauchemin et al., 2003; Shober et al., 2006; Ajiboye et al., 2008; Kruse and Leinweber, 2008;

Kar et al., 2011; Peak et al., 2012). Each chemical species has its own unique spectral characteristics and the spectrum of a mixture of two or more chemical species of an element can be deconvoluted to accurately and quantitatively reflect the amount of each species in the sample being measured. Several studies of soil-fertilizer interactions have used P XANES in this manner. Lombi et al. (2006) used XANES spectroscopy and isotopic dilution method and reported that P precipitated as hydroxyapatite (HAP) in the application zone when mono-ammonium phosphate was applied in granule form to a calcareous soil. Sato et al. (2005) used XANES spectroscopy and reported that both dicalcium phosphate (DCP) and Fe-phosphate were formed over the short-term when poultry manure was applied in a forest soil. Kar et al. (2011) also used XANES spectroscopy and reported that adsorbed and crystalline apatite forms of P were present after long-term field scale broadcast inorganic fertilizer application, whereas a mixture of Al and Fe phosphates and poorly crystalline DCP were formed after broadcast organic fertilizer (biosolids) application.

With advances in equipment and application technology, it is more common for manure and fertilizer P to be injected in soil in bands versus broadcast. This alters the distribution of soil P forms, yet there has been little or no work on the impact of manure and fertilizer P injection in a concentrated band on soil P chemistry under field conditions. Therefore, the goal of this study was to determine the spatial distribution and chemical speciation of soil P months following application when manure or inorganic fertilizer P is placed in the soil in a band in the field under field conditions.

4.3 Materials and Methods

4.3.1 Site description

The experiment was conducted at two field research sites. The site at which the manure

band study was conducted is the Dixon site while the inorganic fertilizer band experimental site is the Central Butte site. The Dixon site is located near the town of Humboldt adjacent to Saskatchewan Provincial Highway #5 (52˚ 12′ 00″ N latitude, 105˚ 07′ 28″ W longitude) in east-central Saskatchewan. The soil at this site is classified as Black Chernozem (equivalent U.S. classification is Udic HaploBoroll) of Cudworth Association, both in the up and downslope positions in the field. The soil is formed in calcareous, silty, lacustrine parent materials and is loam in texture with gently sloping (0-4%) land (Soil Classification Working Group, 1998). The Central Butte site is located near the town of Central Butte (50˚ 47′ 31″ N latitude, 106˚ 30′ 28″ W longitude) in south-central Saskatchewan. The soil at this site is classified as Brown Chernozem (equivalent U.S. classification is Aridic Haploboroll), with Rego Brown Chernozem at upslope positions and Eluviated Brown Chernozem at downslope positions and belonging the Ardill loam Association. The soil at this site is loamy textured with moderately sloping (0-10%) topography (Saskatchewan Soil Survey, 1989).

The selected soil characteristics for both sites were measured by collecting soil samples from upper and downslope positions prior to treatment applications. Soil texture was determined using a HORIBA LA-950 Laser diffraction particle-size analyzer (HORIBA Scientific Inc., Edison, NJ, USA). The pH and electrical conductivity (EC) were measured by glass electrode method using a 1:2 soil/water suspension (Nelson and Sommers, 1982). The organic carbon (OC) of surface soil (0 to 15 cm) was determined using the dry combustion method using LECO-C632 carbon determinator (LECO© Corporation, 1987). Cation exchange capacity (CEC) was measured by 1 *M* NH_4OAc at pH 7.0 (Sumner and Miller, 1996). The amount of available P was determined by a modified Kelowna method (Qian et al., 1994). The selected soil properties are reported in Table 4.1.

In prairie agricultural systems, comparison of soils in a field based upon their landscape position (upslope vs. downslope) is often useful because the upslope soil's physicochemical properties are typically the result of loss of surface soil as a result of historical tillage and water erosion. The upslope position is often characterized by lower organic matter and a carbonate-rich horizon at, or very close to, the surface. In contrast, the downslope position often is where eroded soil, fertilizers, and water collect. These soils are therefore closely linked but variable in physical and chemical properties. To adequately cover processes as they occur in the field, the effect of landscape must be considered.

4.3.2 Experimental design and treatments

Mono-ammonium phosphate (MAP) blended with urea as inorganic fertilizer, and solid cattle manure (SCM) were the fertilizers used in this study. The inorganic fertilizer (MAP) band plot at the Central Butte site was set up with a single application of a blend of urea and mono-ammonium phosphate. The fertilizer was a blend of 66% urea (46-0-0) and 33% mono-ammonium phosphate (12-51-0), giving a blend analysis of 34-17-0 that was applied at a rate of 54 kg N ha^{-1} and 12 kg P ha^{-1}, a typical rate of N and P application for the region. Bands were applied in the field using a John Deere 610 air seeder at 30 cm row spacing with a Dutch Industries banding knife that creates a 2.5 cm wide band at a depth of 10 cm into which the fertilizer is placed. The fertilizer band was applied on May 19, 2009 and samples were collected on June 23, 2009, giving a time period of about one month from the time of fertilizer application to the time of sampling. The field remained fallow in 2009.

The manure band plot in Dixon was set up with two years (May, 2008 and 2009) of band application of SCM and soil samples were collected in June 2009. The SCM was collected from a feedlot in Saskatchewan and homogenized using a rototiller before application.

Table 4.1. Some selected characteristics of soil at the study sites.

Site	Soil	Soil association	Texture	pH	EC† ($mS\ cm^{-1}$)	OC‡ (%)	CEC§ ($cmol\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)
Central Butte	Upslope	Rego Brown Chernozem	Loam	7.9	0.15	0.8	18.2	2.3
	Downslope	Eluviated Brown Chernozem	Loam	5.9	0.12	1.6	31	6.4
Dixon	Upslope	Orthic Black Chernozem	Loam	7.8	0.22	1.92	19	5.6
	Downslope	Orthic Black Chernozem	Loam	6.7	0.27	2.35	24.1	17.7

†EC, electrical conductivity; ‡OC, organic carbon; §CEC, cation exchange capacity

Solid cattle manure samples were collected by sampling at various points of the feedlot stockpile following the Tri-Provincial manure application and sampling guidelines (Saskatchewan Agriculture and Food, 2003). Total P concentration was 2518 mg P kg^{-1} wet manure, measured colorimetrically by Murphy and Riley method (1962) using a Technicon Autoanalyzer II after standard H_2SO_4-H_2O_2 digestion (Thomas et al., 1967). Available P was 193 mg P kg^{-1} wet manure, and measured by modified Kelowna method (Qian et al., 1994). The SCM was subsurface injected in a band at the rate of 60 t ha^{-1} yr^{-1} which is equivalent to 220 kg P ha^{-1}, with a band spacing of 30 cm at a depth of 10 cm. This manure application is typical for western Canada, in which large applications of manure are made that are intended to supply nutrients for a few years before application resumes again. The SCM was subsurface injected using the PAMI Solid Cattle Manure Injector Machine in six subsurface trenches using coulter openers spaced 30 cm apart. The experimental plot was 24.5 m long and 18.5 m wide.

4.3.3 Sample collection

Intact horizontal soil profiles were collected from each site using a nailboard monolith technique developed for intact removal of soil cross-sections. In this method, a trench was excavated approximately 50 cm in depth, 90 cm in length and 80 cm in width. The wall of the trench was sheared to accommodate the nailboard and smoothed to allow a uniform depth of soil to be removed from the trench. The nailboard was inserted into the wall of the trench and the trench wall was backdug to break it off from the soil profile. The nailboard monolith was then gently laid on the board side, removed from the trench, wrapped in plastic and stored at -20˚C. Before sample collection, monoliths were removed from frozen storage and thawed to room temperature. After thawing, soil samples were then collected from each monolith using a micro-coring device starting at the center of the band and at 5 cm intervals away from the band center

to a distance of 20 cm at 10 cm depth. The band was marked in the nailboard at the time of monolith collection from the field. The collected soil samples were air dried, ground to pass a 2-mm sieve, and stored at room temperature for chemical analysis and XANES spectroscopy.

4.3.4 Chemical analysis

After sampling across field-applied fertilizer bands using soil monolith collection, we applied a combination of anion exchange resin membrane probes, sequential chemical extraction, and synchrotron based techniques to perform spatially-resolved P speciation. The amount and form of soil P was assessed both in the application zone and further away after manure and commercial fertilizer P band application. Exchangeable soil labile P supply rates were determined directly in the monolith using anion exchange resin membranes (PRS^{TM} probes) as described by Qian et al. (1992). In this method, after the nailboard monoliths were removed from frozen storage and thawed, anion resin membranes were inserted by creating a narrow opening and inserting to a depth of 10 cm at 5 cm intervals from band center to 20 cm away from the band. Locations were marked to allow for removal of the probes after 24 h. After the 24 h period, they were removed, cleaned with distilled water, eluted using 0.5 *M* HCl, and further shaken for 1 h to desorb the nutrient ions from the anion resin membrane. Sample solutions were stored at 4^{o}C until colorimetric P determination (using ammonium molybdate blue color development) as described by Murphy and Riley (1962). The labile extractable soil P concentration was measured by shaking ion exchange resin membrane in a soil water suspension for one hour according to the procedure by Schoenau and Huang (1991). Briefly, 0.5 g of air-dried soil (collected by micro-coring device from monolith) was placed in a 50 mL centrifuge tube and shaken for 16 h with 30 mL deionized water and a strip of anion exchange membrane. The resin membrane was then removed, placed in 20 mL 0.5 *M* HCl, and shaken for 16 h. The labile P concentration was then

measured colorimetrically (Murphy and Riley, 1962). Total soil P was determined on collected soil samples using a standard H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967) followed by colorimetry (Murphy and Riley,1962).

4.3.5 X-ray absorption near-edge structure analysis

Phosphorus K-edge XANES spectra of the soil samples and P standards were collected at the Soft X-ray Micro-characterization beamline (SXRMB) at the Canadian Light Source (CLS), Saskatoon, Canada. Measurements were performed with InSb(III) monochromator with focused beam size 300µm×300µm. The beamline was calibrated using $ZnPO_4$ powder to the edge energy of 2158 eV in total electron yield spectrum. A small amount of air-dried, ground composite soil sample from each treatment or ground P standards was thinly spread over double-sided C tape, mounted on a stainless steel sample holder and placed in the vacuum chamber. Soil sample spectra were collected in partial fluorescence yield (PFY) mode to improve detection limits over total fluorescence or electron yield measurements, and spectra of P reference standards were collected in total electron yield (TEY) mode to avoid self-absorption effects due to high concentration (Toor et al., 2005). PFY uses an energy-discriminating solid-state detector to select fluorescence arising from P from the full range of emitted photon energies (Achkar et al., 2011); this detection method is suitable for bulk measurements of dilute samples (Achkar et al., 2011; Tamenori, 2011). Spectra were collected from 2135-2190 eV with a step size of 1 eV on the pre edge region (2110-2145 eV), 0.25 eV in near edge region (2145.25-2180 eV), and 0.5 eV in post edge region (2180.5-2200 eV) with a constant dwell time of 4.0 s. Multiple spectra were collected and averaged as required to obtain an adequate signal to noise ratio for analysis. XANES spectra were analyzed using WinXAS 3.1. software.

The averaged XANES spectra were background subtracted to the pre-edge region (2,135-2,145 eV) using a first-order polynomial fit and were normalized to an edge jump of 1.0 between 2,140 and 2,180 eV. Linear combination (LC) fitting was performed over the spectral energy range of 2,145 to 2,180 eV. This large energy range was used to include more chemical information and therefore increase the probability that similar standards can be properly distinguished (Toor et al., 2005; Peak et al., 2012). The initial E_0 value was allowed to vary to provide an initial estimate of percent contribution, but in the second iteration E_0 values were fixed to ‘‘0’’ for all components since the beamline had been calibrated to a $ZnPO_4$ standard regularly during data collection. The fit with the *F* tests (probability of *F* values) was chosen as the best fit in most cases in WinXAS’s fit routine which provides statistical basis to reject or accept a given standard.

4.3.6 Phosphorus XANES standards

The P standards used in this study were purchased as reagent grade powders: zinc phosphate ($ZnPO_4$), brushite ($CaHPO_4.2H_2O$), berlinite ($AlPO_4$), ammonium phosphate monobasic ($NH_4H_2PO_4$), apatite [$Ca_5(PO_4)_3(OH,F,Cl)$], strengite ($FePO_4.2H_2O$), phytic acid, adenosine $5^{//}$-monophosphate (AMP), adenosine $5^{//}$-diphosphate (ADP), adenosine $5^{//}$-triphosphate (ATP), deoxyribonucleic acid (DNA). A sample of phosphate adsorbed on an Fe oxide (HFO) at pH 6 was synthesized in the lab in a previous study (Peak et al., 2012).

4.3.7 Sequential chemical extractions of phosphorus

Different forms of P in the center of the band were determined by the sequential chemical extraction method described by Tiessen and Moir (1993). For the sequential extractions, 0.5 g of air-dried soil was first extracted with 30 mL of deionized water and a strip of anion exchange membrane, followed sequentially by 0.5 M NaHCO3 (pH 8.5), 0.1 M NaOH, and 1.0 M HCl.

For resin extraction, the resin membrane was removed from soil suspension and shaken with 20 mL 0.5 *M* HCl for 16 h in rotary shaker. After each extraction, the soil and water was separated via centrifugation for 10 min at 8,000 x g at 0˚C, and the supernatant was then filtered without disturbing the sedimented organic matter using a 0.45-µm filter. This extraction process was repeated for 16 h in a rotary shaker for each extraction. The remaining soil residue from all soil samples was digested using a mixture of 30% H_2O_2 and concentrated H_2SO_4 to extract any residual P that remained (Thomas et al., 1967).

Total extractable P was determined from each step of the extraction except resin extraction using the following procedure. A 10 mL filtered extractant from each soil sample was placed into a 50 mL volumetric flask and then 0.5 g of ammonium persulfate and 10 mL of 0.9 *M* H_2SO_4 were added to each flask. The flasks were gently boiled placing on a hot plate until white fumes appeared. At this point, about 0.3 mL of solution remained in the flask. Then the flasks were removed from the hot plate and allowed to cool to room temperature. Then 10 mL deionized water was added each flask after cooling. The pH was adjusted using 4 *M* NaOH and 0.25 *M* H_2SO_4 solution and the total P was determined using the Murphy Riley method (1962). Absorbance was determined on Beckman DU-65 spectrophotometer at a wavelength of 712 nm.

4.3.8 Statistical analysis

Statistical analysis was performed using the data analysis package in Microsoft Excel 2007 (Microsoft, Seattle, WA) to calculate mean and standard deviation. The significance of slope position effects on sequentially extracted total P was determined at $P \leq 0.05$ probability levels and was achieved by Student's *t* test using Statistical Package of Social Science (SPSS Inc.).

4.4 Results and Discussion

4.4.1 Soil labile phosphorus supply and total phosphorus

The labile P supply rates and total P concentration in soil samples are shown in Figure 4.1. Overall the labile P supply and total P concentration were highest in the center of the band and decreased rapidly with distance away from the band in both MAP and SCM amended soils (Figure 4.1). Several researchers have shown that P movement from the point of application is limited to about 3 to 5 cm in short-term periods (1 to 3 months) (Blanchar and Caldwell, 1966; Khasawneh et al., 1974; Eghball and Sander, 1989; Eghball et al., 1990), which is similar to the results obtained in this study. Soil amended with the MAP and SCM had higher labile P supply and total P in the downslope position than upslope position (Figure 4.1) which agrees with Schoenau et al. (1989) who similarly reported that total P and soluble P concentrations were higher in downslope positions. Halbfass and Grunewald (2003) also reported that total P and soluble P concentrations were higher in downslope positions than in the upslope positions in their study in agricultural soils in Saxony, Germany. The relationship between total P and higher organic matter content (2.75 % in Central Butte and 4.05 % in Dixon site) in the downslope areas could be due to a combination of direct effects such as the release of P from organic matter over time producing more labile P or more P desorption by competing organic constituents. Organic constituents may also inhibit formation of thermodynamically stable Ca-P minerals keeping higher available P level in downslope soils by complexation of soluble Ca with organic ligands (Grossl and Inskeep, 1992) and blocking soil P adsorption sites (Kashem et al., 2004). This also may be caused by physical movement of labile P from upslope soil by runoff and erosion and accumulation in the downslope position over time. In MAP amended soil, a large difference in P supply rate was observed between up and downslope positions, with resulting rates being much

lower in upslope position (Fig. 4.1A). The soil texture, $CaCO_3$, and Al and Fe contents influence precipitation and sorption reactions in soil (Sharpley et al., 1989). The MAP applied upslope soil at Central Butte has a high pH and free lime content which would reduce P solubility and mobility by promoting formation of more stable Ca-P minerals.

In SCM amended soil, a difference in total P levels between the band center and further away was observed, but P supply rates were similar for both upslope and downslope positions (Fig. 4.1C). This may reflect less difference in pH and soil carbon between up and downslope at the Dixon site compared to Central Butte (Table 4.1) and less interaction of manure P with soil constituents.

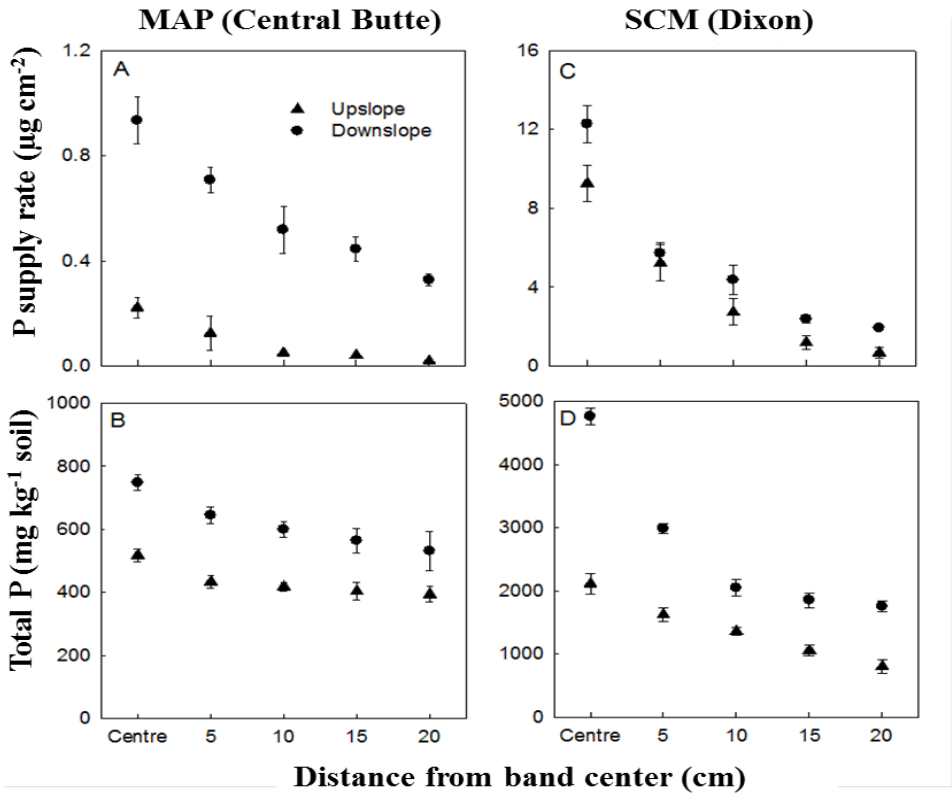

Figure 4.1. Available P supply rate and total P concentration from upslope and downslope positions of (A and B) mono-ammonium phosphate blended with urea (MAP) and (C and D) solid cattle manure (SCM) applied soils as a function of slope position, and distance from injection zone to 20 cm distance from band center. Vertical bars indicate the standard deviation of the mean (n = 3).

4.4.2 XANES analysis

Phosphorus K-XANES spectra of the different phosphate standard compounds used in LC fitting are shown in Figure 4.2. The spectra are normalized and offset for comparison. The XANES spectra of organic and inorganic phosphate standards in Figure 4.2 had main white line peak and a broader peak at around 2169 eV because of a 1s→3p electron transition and oxygen oscillation, respectively (Kruse and Leinweber, 2008). The XANES spectrum of mono-ammonium phosphate showed intense white line peak and phytic acid had a short and broader white line peak. The XANES spectra of different inorganic phosphate standard compounds (Figure 4.2B) exhibited characteristic features in main peak position, number and position of pre-edge and post-edge peaks that can be used for LC fitting analysis. Calcium-phosphate mineral standards exhibited a post edge shoulder on the high-energy side of the absorption edge between 2156 and 2166 eV, and this feature is more prominent with compounds containing more Ca atoms (Hesterberg et al., 1999) and is increased in intensity with increasing crystallinity (Khatiwada, 2011). For example, apatite had a more pronounced shoulder than brushite (Figure 4.2B). The XANES spectra of the Fe-phosphate mineral (strengite) had characteristic pre-edge feature around 2148 eV that increased with increasing crystallinity. The XANES spectra of Al-phosphate (berlinite) and NH_4-phosphate (mono-ammonium phosphate) showed very weak pre-edge inflection at about 2152 eV and shorter white line peak. The XANES spectrum of Zn-phosphate ($ZnPO_4$) had very intense white line peak and very weak post edge shoulder on the high-energy side of the absorption edge around 2162 eV. These spectral features of different calcium, aluminum, and iron phosphate minerals have been observed by many researchers (Hesterberg et al., 1999; Peak et al., 2002; Beauchemin et al., 2003; Khare et al., 2005; Lombi et

al., 2006; Kruse and Leinweber, 2008) and can be used to differentiate different P species formed after the application of organic and inorganic P fertilizer in soil samples.

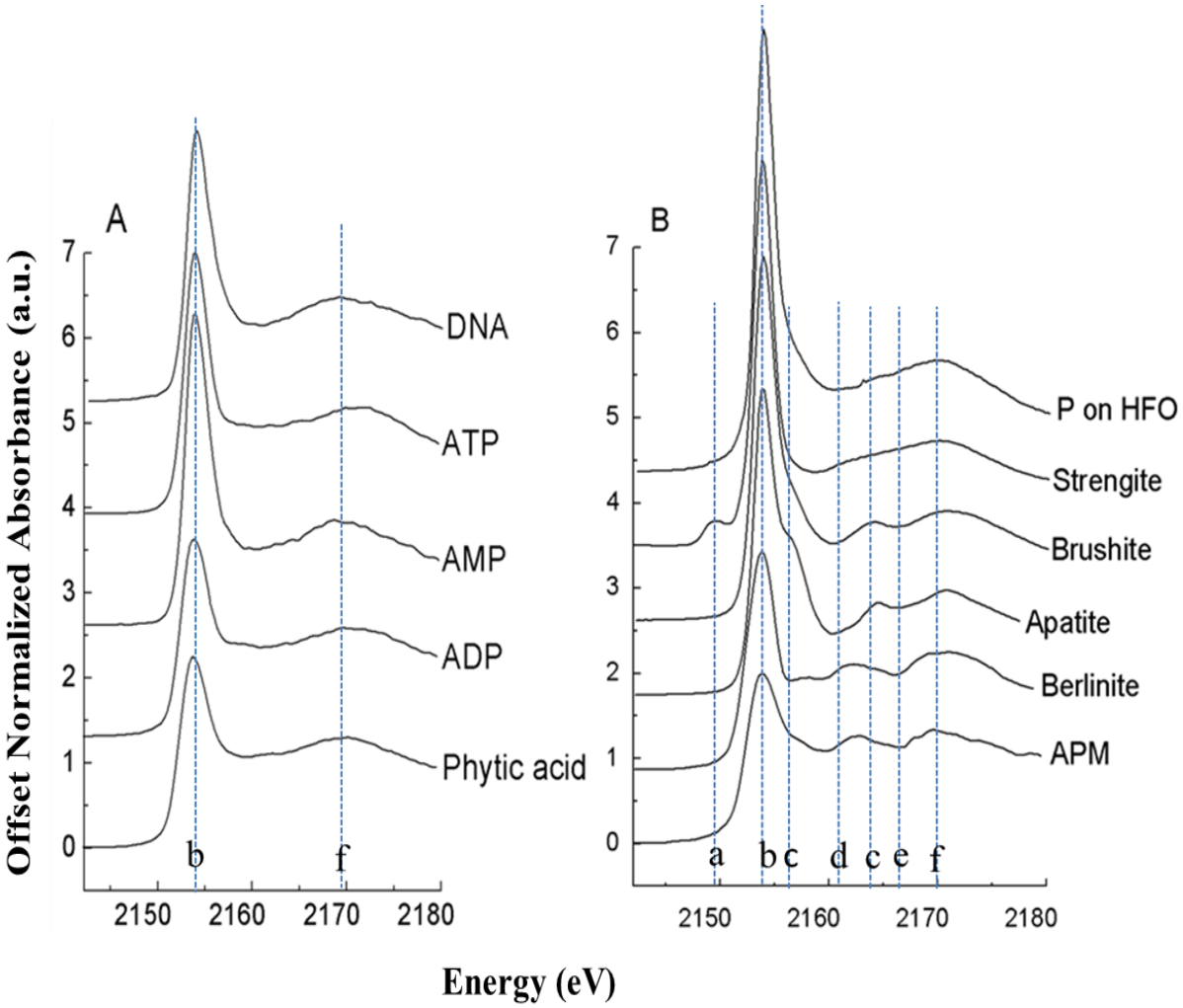

Figure 4.2. Normalized P K-edge X-ray absorption near edge structure (XANES) spectra of different (A) organic P standards: deoxyribonucleic acid (DNA), adenosine 5$^{//}$-triphosphate (ATP), adenosine 5$^{//}$-monophosphate (AMP), adenosine 5$^{//}$-diphosphate (ADP), phytic acid and (B) inorganic P standards: phosphate adsorbed on an Fe oxide (P on HFO), strengite ($FePO_4.2H_2O$), brushite ($CaHPO_4.2H_2O$), apatite [$Ca_5(PO_4)_3(OH,F,Cl)$], berlinite ($AlPO_4$), APM ($NH_4H_2PO_4$) used in linear combination (LC) fitting. Spectra are offset by one absorbance units to allow for comparison of spectral features. The dashed lines show unique spectral features for different P species: the pre-edge of (a) Fe-phosphate, (b) white line peak, (c) unique spectral features of Ca-phosphate, (e, d) unique spectral features of Al and NH_4-phosphates, and (f) oxygen oscillation.

Background-subtracted P XANES spectra of collected soil samples are shown in Figure 4.3. The relative peak height intensity of these background-subtracted spectra corresponded to the total amount of P present in the samples (Figure 4.3E) indicating that there was no distortion from self-absorption and that all our samples are both above detection limit and within the linear range of the absorbance-concentration relationship (Beer-Lambert equation). This provides strong evidence that relative percent changes in the speciation correspond to real changes in soil P distribution. Peak intensity differences among the sample spectra were less in upslope positions (Figure 4.3A and C), whereas the peak intensity drastically decreased between sample spectra collected from center of the band and 5 cm further away from the band center in downslope positions from both treatments (Figure 4.3B and D).

Representative results of LC fitting of P XANES spectra using the MAP upslope and downslope soils at different distances from the center are shown in Figure 4.4. The LC fitting results for each phosphate species in soil samples collected from both slope positions are shown in Table 4.2. For the upslope MAP sample, the P XANES spectra at all distances can be reproduced using a simple two component model: at the center of the band, the majority of P was apatite (63%) with a smaller adsorbed P (37%) component and the proportion of apatite type P increases with distance from the center (Figure 4.4A, Table 4.2). This soil had higher Ca:P ratio, higher pH, and low organic matter content which could favour formation of apatite (Lindsay, 1979), and the proportion of apatite increased with distance because decreased P levels will increase the effective Ca:P ratio and thus favour formation of apatite (Toor et al., 2005). It has also been shown that the presence of high amounts of exchangeable soil Ca promote insoluble Ca-P mineral precipitation (Tunesi et al., 1999; Hansen and Strawn, 2003). This is consistent with the theory that Ca is the predominant factor for P speciation in neutral to alkaline

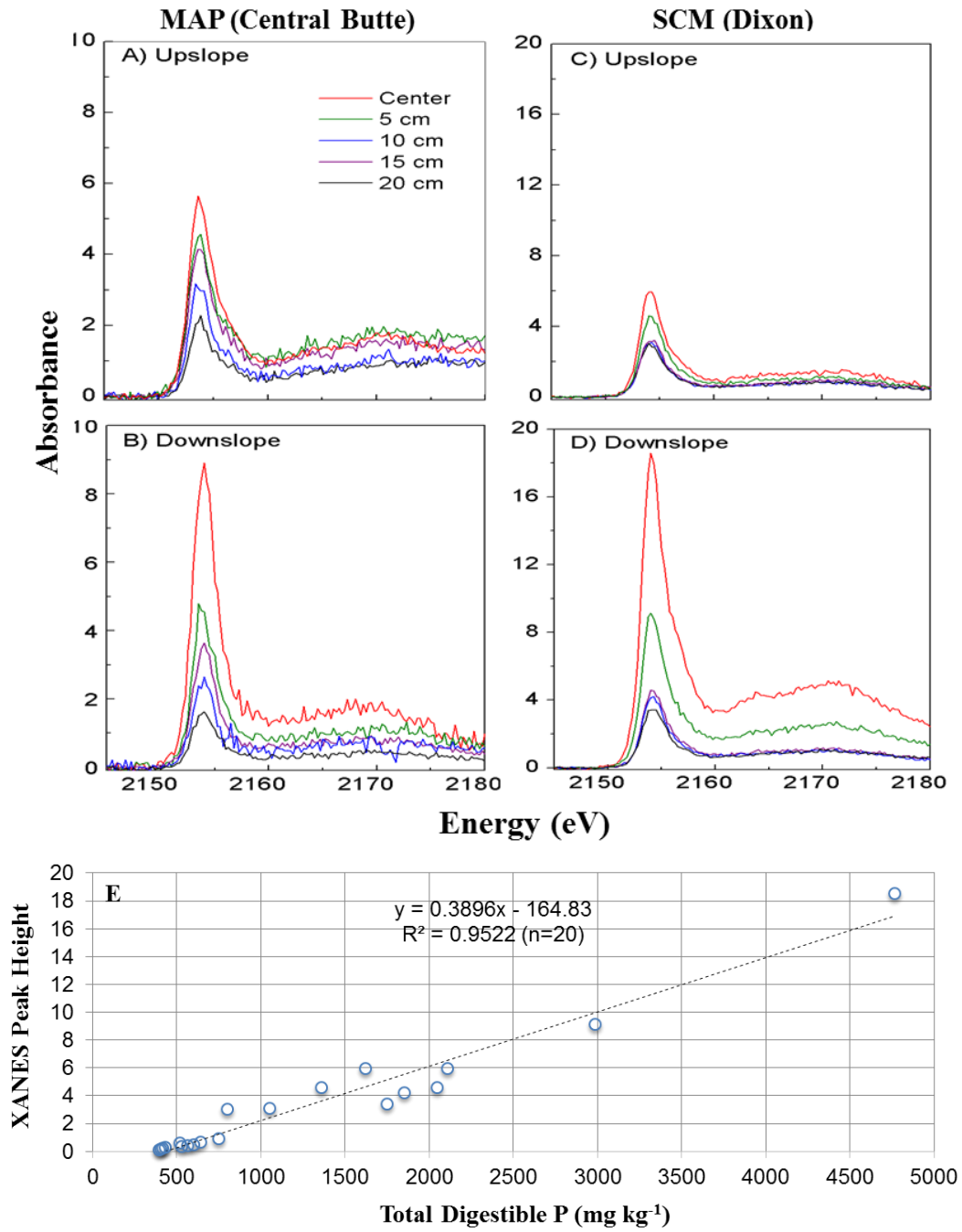

Figure 4.3. Background-subtracted P K-edge XANES spectra on P distribution in [(A) upslope and (B) downslope] fertilized soil (MAP) and [(C) upslope and (D) downslope] manured soil (SCM) as a function of slope position and distance from injection zone to 20 cm distance from band center. Spectra of soil samples collected (from top) center of band, and 5, 10, 15, 20 cm distance away from band. (E) Relationship between XANES peak intensity at the white line (2158 eV) versus total digestible P for all MAP and SCM applied up and Downslope samples in (A), (B), (C), and (D).

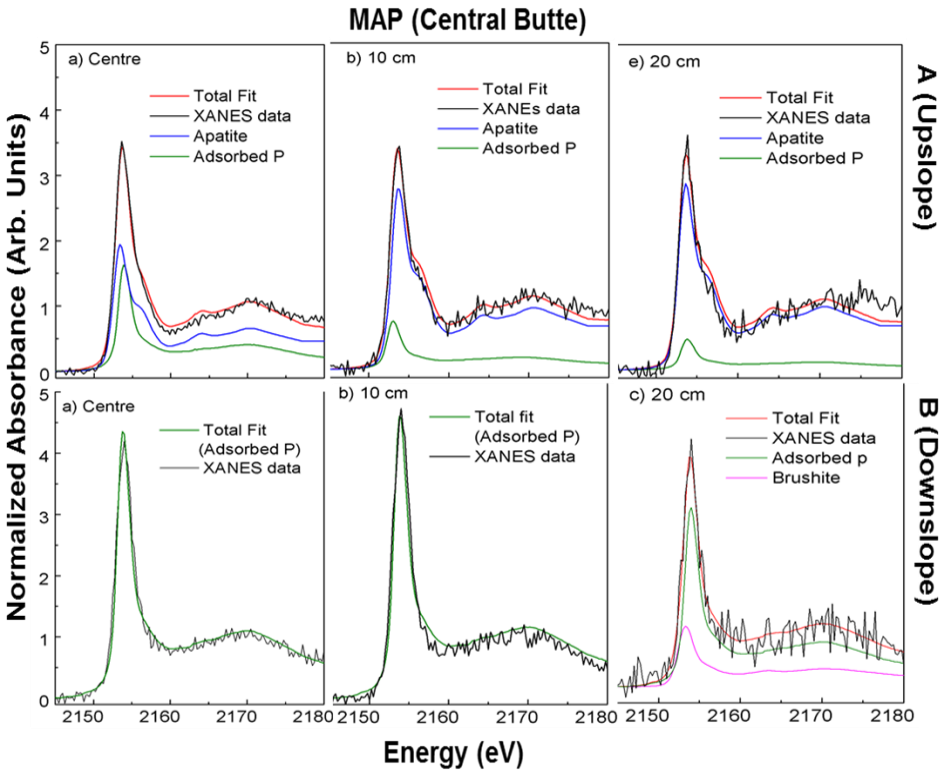

Figure 4.4. Linear Combination XANES fit of differential XANES spectra of fertilized (MAP) upslope (A) and downslope (B) soils: (a) center of the band, (b) 10 cm distance, and (c) 20 cm distance from center of the band.

soils (Lindsay, 1979; Pierzynski et al., 2000). The second dominant species found in this soil was adsorbed P, a proportion that decreased with distance from the band. The likely explanation for this is that decreased pH at the center of the band from nitrification could enhance adsorption of P on Fe-oxide surfaces. This acidity would tend to be confined to the center band area, as soil carbonate will neutralize it as it diffuses outward. The decrease in soil pH after fertilizer application has been reported by many researchers (Hanson and Westfall, 1985; Moody et al., 1995). The LC fits of fertilizer applied in the downslope position (Figure 4.4B, Table 4.2) revealed that P was mainly present as adsorbed P at the center and adjacent to the band center and a smaller amount of P was found as apatite (7%) or brushite (20%), with calcium phosphate and amounts of crystallinity increasing with distance as the Ca:P ratio increased. The presence of

Table 4.2. Linear combination phosphorus X-ray absorption near edge structure (P XANES) fitting results and labile P fractions showing proportions of P compounds in the inorganic fertilized and manured soils.

Soil sample	Ca:P ratio	Labile P†	Brushite	Apatite	Adsorbed P	Organic P
		%				
				MAP††		
Upslope						
Centre	8.6	0.3	-	63	37	-
5 cm	10.1	0.2	-	55	45	-
10 cm	10.7	0.08	-	84	16	-
15 cm	11.5	0.07	-	80	20	-
20 cm	12.0	0.04	-	90	10	-
Downslope						
Centre	2.5	0.87	-	-	100	-
5 cm	2.9	0.8	-	-	100	-
10 cm	3.4	0.6	-	-	100	-
15 cm	3.9	0.55	-	7	93	-
20 cm	4.6	0.43	20	-	80	-
				SCM‡		
Upslope						
Centre	1.7	3.08	60	-	-	40
5 cm	2.3	2.3	44	-	-	39
10 cm	2.6	1.4	45	-	55	-
15 cm	3.2	0.8	27	-	73	-
20 cm	5.2	0.58	-	47	53	-
Downslope						
Centre	0.7	1.8	75	-	-	25
5 cm	0.8	1.34	46	-	54	-
10 cm	1.3	1.50	11	-	89	-
15 cm	1.6	0.90	9	-	91	-
20 cm	2.2	0.80	42	-	58	-

††MAP, mono ammonium phosphate blended with urea applied soil

‡SCM, solid cattle manure applied soil

†Labile P percentage was calculated as Resin extractable P/total P

organic acids and Al and Fe oxides in downslope soil as reported by Schoenau and Bettany (1987, 1989) may inhibit the precipitation of more stable Ca-P minerals like HAP by lowering the free Ca^{+2} concentration in solution as a result of complexation of soluble Ca^{+2} by organic

ligands (Grossl and Inskeep, 1992; Hansen and Strawn, 2003). Since the pH was lower in the downslope position, available P was present as adsorbed form on metal oxide surfaces. Thus, the proportion of apatite and adsorbed P in MAP treated soil suggests that a combination of adsorption and precipitation were dominant retention mechanisms at the center and adjacent to the center, while precipitation was dominant retention mechanism further away from center. The dominance of adsorbed P in downslope soils suggests that adsorption may be the main retention mechanism throughout.

The fitting results for SCM treated soil (Figure 4.5 and Table 4.2) showed that two major species of P were present in SCM treated upslope soils. The spectra of center of the band and nearby suggested that P was mainly present as brushite (60%) and organic P (40%). Further away from the band, the soils contained adsorbed P and apatite (Figure 4.5A and Table 4.2). The Ca:P ratio was lower (1.7:1) in the band area and it increased with increasing distance. This may be the reason for the predominance of brushite (dicalcium phosphate) in the center and adjacent to the center samples. The presence of organic P in the SCM treated soil is expected because SCM contains a significant amount of organic P from feed passing through the animal and due to the mixing of straw bedding materials during production (Ajiboye et al., 2004). The major effect observed was that organic P was localized near the band. Adsorbed P and apatite dominated further away from band as Ca:P ratio increased.

The spectra of the downslope position SCM amended soil (Figure 4.5B) indicated that the majority of P was present as brushite (75%) and organic P (25%) accounted for the rest at the center of the band. Near the band center, the majority of P was still present as brushite (62%) and rest of the P was adsorbed and organic P, which were 20% and 18%, respectively.

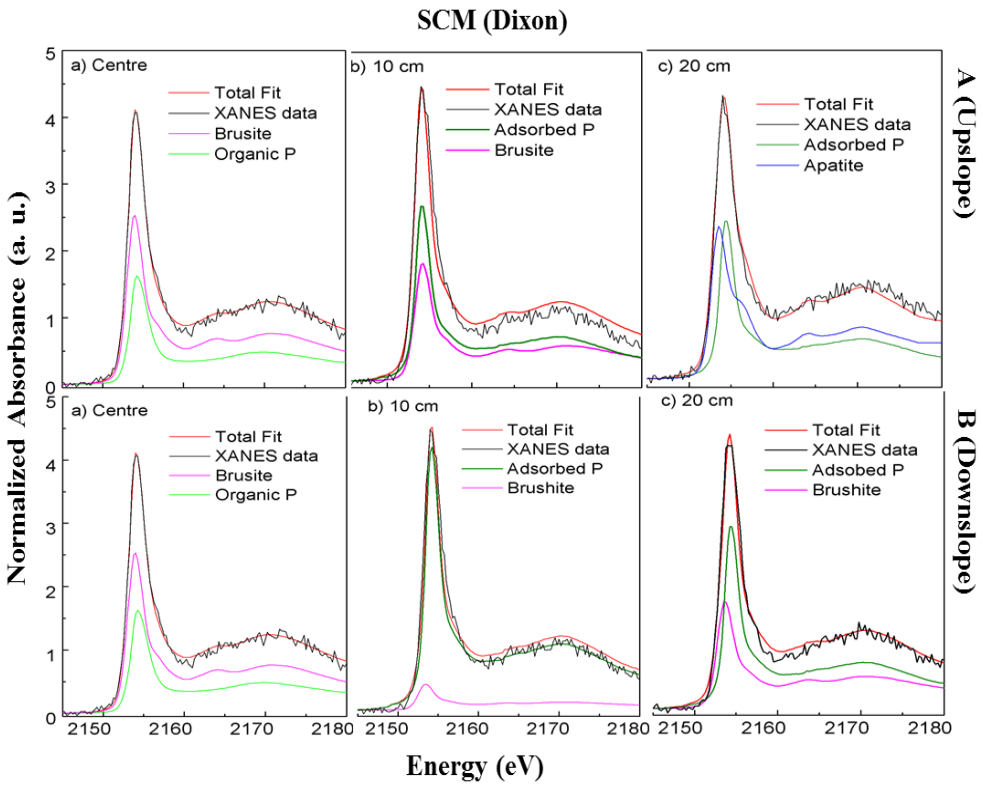

Figure 4.5. Linear Combination XANES fit of differential XANES spectra of manured (SCM) upslope (A) and downslope (B) soils: (a) center of the band, (b) 10 cm distance, and (c) 20 cm distance from center of the band.

At further distances from the band center, the soils were dominated by adsorbed P with a minor brushite component (Figure 4.5B and Table 4.2). In all downslope samples, the Ca:P ratio was much lower (less than 1) compared to the SCM upslope or either of the MAP amended soils at Central Butte site, which may be the reason for the higher proportion of brushite in downslope soil samples at Dixon site. This is consistent with the results reported by Toor et al. (2005) where an observed higher proportion of dicalcium phosphate in broiler litter and manure samples when Ca to P ratio was low (less than 2). Another possible reason for the predominance of brushite in the band center in manure amended calcareous soil is that organic acids in the manure inhibit the precipitation of more stable Ca-P minerals (Grossl and Inskeep, 1992; Laboski and Lamb, 2003). For both landscape positions, organic P was localized near the band. The predominance of

adsorbed P with increasing distance from the band and the dominance of brushite and adsorbed P in both up and downslope soils implies that that desorption/adsorption may be the main retention mechanism in SCM treated soils.

XANES spectroscopy did not identify any organic P species further than 5 cm away from the center for any SCM soil samples. Many researchers (Beauchemin et al., 2003; Sato et al., 2005; Ajiboye et al., 2008) have reported that organic P as phytic acid was not detected by XANES in their bulk soils; our results imply that this may be due not only to difficulty in assignment of organic P via K-edge XANES but also due to localization of organic P derived from manure within the injection band.

4.4.3 Soil phosphorus fractionation

Chemical extraction of soil P from the center of the band showed that slope position had a strong effect on the distribution of different P fractions in both treatments (Table 4.3). Total P was significantly higher in downslope than upslope position for both MAP and SCM amended soils. The P redistribution to the downslope areas occurs via runoff and erosion over several years. In MAP treated soil, labile P (operationally defined as resin P + $NaHCO_3$-P) accounted for 24% in upslope soil and for 47% in downslope soil. The largest P fraction was found in non-labile fractions (HCl extraction) in upslope position but the largest P fraction was removed by $NaHCO_3$ and NaOH in downslope position. The results from the upslope soil suggest that P was mainly associated with crystalline $CaPO_4$ minerals in the calcareous upslope soil (63% of total P as HCl-P), whereas the labile and Al and Fe oxide associated P fraction (adsorbed P) represented the major forms of P minerals in downslope soil (67% of total P as $NaHCO_3$-P and NaOH-P). This agrees with the XANES analysis results.

Table 4.3. Sequentially extracted P fractions from upslope and downslope positions of mono-ammonium phosphate (MAP) blended with urea and solid cattle manure (SCM) banded soils in the center of the band (mean ± standard deviation).

Soil	Resin-P	$NaHCO_3$-P	NaOH-P	HCl-P	Residual-P	Total P
	mg P kg^{-1}					
			MAP†			
Upslope soil	23±1(5)¶	93±13(19)	38±4(8)	312±28(63)	31±1(6)	498a
Downslope soil	75±7(11)	206±3(31)	222±37(33)	142±5(21)	26±2(4)	673b
			SCM‡			
Upslope soil	67±16(3)	1046±81(49)	701±16(33)	212±9(10)	98±11(5)	2124a
Downslope soil	161±4(3)	2629±136(56)	1658±65(35)	119±11(3)	143±19(3)	4710b

†MAP, mono-ammonium phosphate blended with urea; ‡SCM, solid cattle manure

¶The numbers in parentheses are the fraction as a percentage of total P

§Means followed by different letters for same treatment are significantly different at $P \leq 0.05$ level for each treatment

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In this MAP treated soil, pH will decrease on application due to a combination of factors: both the dissociation and hydrolysis of MAP, and acidity produced from nitrification of the ammonium originating from MAP and urea. This decreased pH would enhance P sorption on variably charged soil surfaces (edges of clay minerals and iron oxides) but in the upslope soil, the buffering of soil carbonates will raise the pH and ultimately favour the formation of less soluble calcium phosphate minerals. Lindsay (1979) also observed that formation of labile P from inorganic fertilizer P is limited in calcareous soils due to formation of sparingly soluble Ca minerals because higher amounts of exchangeable Ca promote the formation of insoluble Ca-P precipitates which control P availability (Tunesi et al., 1999). Since the downslope soil is more leached, has a lower Ca:P ratio, and lower pH, it is reasonable that an increase in P association with Al and Fe oxides (operationally defined as the NaOH extractable inorganic P) might increase compared to the upslope (Schoenau et al., 1989).

In SCM applied soil, labile P (resin P + $NaHCO_3$-P) accounted for 52% in upslope and 59% of total P in downslope soil. The largest P fraction was found in labile ($NaHCO_3$-P) and moderately labile fractions (NaOH-P) in both slope positions but the labile and moderately labile fractions were much higher in downslope position, whereas HCl extractable and residual P fractions were higher in upslope position (Table 4.3). In both slope soil samples in SCM treated soil, most of the P fractions were associated with poorly crystalline Ca-bound and adsorbed P minerals (82% in upslope and 91% in downslope position of total P). Similar to our results, Laboski and Lamb (2003) reported enhanced availability of P in manure amended calcareous soil, and attributed it to organic acids in the manure that prevented P precipitation in the soils and enhanced P availability via formation of poorly crystalline Ca-bound and adsorbed P minerals. Higher amounts of labile and moderately labile P fractions were found in downslope (4287 mg P

kg^{-1}) than upslope soil samples (1747 mg P kg^{-1}). Ajiboye et al. (2008) reported that majority of P was present as adsorbed form in manure amended soil. The lower amount of labile and moderately labile P fractions in upslope soil than downslope soil could be due to available P losses by runoff and erosion in upslope positions. The higher amount of HCl extractable and residual P fraction which is mainly stable Ca-P minerals, indicates that higher Ca:P ratio and high pH favoured formation of more stable apatite like P minerals at upslope soil as shown in the XANES analysis.

4.5 Conclusions

Band application of inorganic P fertilizer or cattle manure increased the total P concentration and the labile P supply at the center of the band; the magnitude of the increase was much higher in the downslope position of the landscape. The labile P supply rate in the inorganic P fertilizer band center was more than two times higher than in soil 10 cm away from the band center, confirming that banded P was confined to a relatively small area. Labile and moderately labile P fractions dominate in downslope positions while more stable calcium phosphate minerals dominate in upslope positions of the landscape. The quantitative XANES fitting showed that a combination of adsorption and precipitation were dominant retention mechanisms at the band center while precipitation reactions were dominant further away from band center and also confirmed that adsorption is the main retention mechanism in downslope position in MAP treated soils. The dominance of brushite and adsorbed P in both up and downslope soils suggests that adsorption/desorption is the main retention mechanism in SCM treated soils. The LC fitting results also indicated that organic P had very limited movement and was always localized near the manure band. A greater preponderance of more labile inorganic P forms in low slope

positions of amended soils suggests that these soils may pose greater risk for further movement of soluble reactive phosphate off-site with water leaving these regions of the landscape.

CHAPTER 5.0

PHOSPHORUS DISTRIBUTION AND CHEMICAL SPECIATION IN SASKATCHEWAN SOILS AMENDED WITH MANURE FOR ONE AND ELEVEN YEARS

5.1 Preface

In the preceding two chapters the nature of soil P in the weeks and months following application of P were examined. This chapter covers the fate of manure P over longer duration and with several years of repeated application of LHM and SCM at low and high rates. Soil P speciation is revealed in manured soils over short (1 to 2 years) and longer term (11 years) application histories using wet chemical methods and synchrotron spectroscopy.

5.2 Introduction

Livestock manures are valuable source of nitrogen (N) and phosphorus (P) which can benefit crop production (Kashem et al., 2004). However, continuous, repeated application of manures based on the crop N requirements to agricultural land is being currently practiced in some regions of the Canadian Prairies (Simard et al., 1995; Whalen and Chang, 2001; Ajiboye et al., 2004) and can increase the accumulation of P in soil.

Variation in P fixation and availability associated with different manure sources (e.g. hog, cattle) and differences in soil properties and management practices make it difficult to predict potential loss of manure P to surface and groundwater without assessment of soil P forms (Stumborg and Schoenau, 2008). Phosphorus is mainly present as soluble and insoluble orthophosphate forms in soil (Frossard et al., 2000), and water extractable P is an indicator of P that is susceptible to runoff and leaching (Sharpley and Moyer, 2000). Some researchers (Ajiboye et al., 2004; Brandt et al., 2004; Kashem et al., 2004; Kleinman et al., 2005) have

shown that the ratio of water extractable to total P in manure itself can vary depending upon the manure type. Tunney and Pommel (1987) reported that P in pig slurry contains 70 to 90% P_i while some manures contain more P_o. Other studies have shown that manure P may be equally or more available than fertilizer P (Meek et al., 1979; Gale et al., 2000). Kashem et al. (2004) showed that the effects of manure on P distribution in soil vary with the type of animal manures due to different P concentrations and forms in animal feeds, differences in composition, storage, and application methods and stage of decomposition. Qian et al. (2004) also have shown that soil total P and labile P significantly increased after cattle manure application for five years but no significant increase was observed after liquid swine manure application for the same period of time. In particular, labile P fractions significantly increased with increasing rate of cattle manure addition.

High rates of livestock manure or fertilizer P application alter the amounts and distribution of P in the various pools of soil P (Campbell et al., 1986; O'Halloran, 1993; Zheng et al., 2001). Dormaar and Chang (1995) showed that the H_2O-extractable P_i and the $NaHCO_3$-extractable P_i and P_o increased in soil after 20 annual applications of feedlot cattle manure in a southern Alberta soil. On the other hand, Qian and Schoenau (2000a) reported that single addition (one year) of liquid swine manure to an Orthic Black Chernozem did not increase the labile soil P fractions. Working in Quebec, Simard et al. (2001) showed that the application of liquid swine manure produced a rapid increase in anion exchange membrane extractable P. Zheng et al. (2004) carried out a field experiment with continuous application of fertilizer-P and liquid dairy manure for eight years at the rate of 20 kg P ha^{-1} yr^{-1} and reported that resin P increased by 2.6 to 6.3 mg P kg^{-1} and the long-term (10 years+) application substantially increased the labile P fractions (Richards et al., 1995). Condron and Goh (1989) reported that the

absolute concentration of the labile and moderately labile P_i fractions had been doubled after the application of dairy manure at the rate of 62 kg P ha^{-1} yr^{-1} for 19 years. Continuous long-term applications from different sources can increase the concentration of labile and moderately labile P fractions even when the application rate is low (Wakene and Peter, 2009).

Several soil test phosphorus (STP) methods are used to estimate available or labile soil P for agronomic and environmental assessment purposes. Several studies (Pote et al., 1999; Pierson et al., 2001; Fang et al., 2002) have shown that there is direct relationship between STP and P concentrations in overland flow from both non-calcareous and calcareous soil, which is useful for environmental purposes. Agronomic tests such as Olsen, modified Kelowna method are useful to estimate the available P to plant throughout the growing season (Olsen et al., 1954; Van Lierop, 1988; Wright et al., 2003). Environmental soil P tests such as distilled water extraction are used to estimate the mobile P fractions (Atia and Mallarino, 2002) and have shown high correlation with dissolved P in runoff (Pote et al., 1996).

Despite their utility, while various STP methods can quantify the available or potentially mobile P forms, they cannot identify a particular chemical species present in soil (Hunger et al., 2005; Toor et al., 2005). There is a need to identify specific chemical forms of P in soil as well as operationally defined labile P pools revealed by different soil test methods. Knowing specific P chemical forms in manured soils can aid in identifying management practices that can help increase plant utilization of P and reduce the losses to runoff water. For example, knowing that Ca-P compounds are dominant in specific manure types and in the soils receiving these manures would be helpful in understanding how practices that increase soil acidity such as adding N fertilizer could potentially influence the solubility of P. Therefore, further studies are needed to

identify specific chemical forms of P. Using a molecular-scale speciation technique such as P K-edge X-ray absorption near-edge structure (XANES) spectroscopy is one useful approach.

X-ray absorption near-edge structure spectroscopy is a molecular-scale analytical technique and it has been used for speciation of P minerals present in manure and manure amended soil (Sato et al., 2005; Shober et al., 2006; Gungor et al., 2007; Ajiboye et al., 2008), poultry litter (Peak et al., 2002; Toor et al., 2005), organic amendments (Ajiboye et al., 2008; Kar et al., 2011; Peak et al., 2012), and agricultural byproducts (Kruse et al., 2010). XANES spectroscopy is a non-invasive element specific technique (Gungor et al., 2007) and is sensitive to the local chemical and structural environment of the element as well as the oxidation state (Fendorf and Sparks, 1996; Khatiwada, 2011). XANES linear combination (LC) fitting has been used to quantify the relative proportions of P minerals present in manure and manure amended soil samples. In this technique, the P K-edge XANES spectrum of the unknown sample (soil sample) is compared with a library of known P standard spectra to examine the similarities (Fendorf and Sparks, 1996) and many researchers (Beauchemin et al., 2003; Sato et al., 2005; Toor et al., 2005; Ajiboye et al., 2008; Kruse et al., 2010; Kar et al., 2011; Peak et al., 2012) have used this technique to investigate P speciation. Kar et al. (2011) used XANES spectroscopy and reported that both adsorbed and crystalline apatite forms of P were present after long-term broadcast application of inorganic P fertilizer, whereas a mixture of aluminum and iron phosphates and poorly crystalline dicalcium phosphate (DCP) were formed after broadcast application of biosolids. Sato et al. (2005) used XANES spectroscopy to assess forms of Ca-P minerals formed in poultry manure amended acidic forest soils, and reported that both DCP and Fe phosphate were formed over the short-term and a more stable form of Ca phosphate (tri-calcium phosphate) was formed over longer time periods.

An *in situ* identification of P species formed in soil receiving different manure application treatments combined with wet chemical analysis should ultimately provide a better understanding of P transformations and the bioavailability and potential for loss of P following manure application to agricultural soils. Therefore, the objectives of this study were to 1) evaluate the effects of long-term (11 years) and short-term (1-2 years) manure application on the amounts and distribution of labile and total P in soils and 2) identify P species or P reaction products formed in the manure amended soils using XANES spectroscopy. The soil samples used in this study were collected from well documented field studies that have received both long- and short-term application of SCM and LHM at different rates.

5.3 Materials and Methods

5.3.1 Site description

The experiment was conducted near Dixon in the province of Saskatchewan, Canada at legal location NW21-37-23-W2 in east-central Saskatchewan. The soil at this site is classified as Black Chernozem (Udic Boroll) of Cudworth Association. The soil is formed in calcareous, silty, lacustrine parent materials and is loam surface texture (0 to 15 cm) with gently sloping (0-2%) topography (Saskatchewan Soil Survey, 1989).

The study site includes four different experiments. The experiments were long-term (11 years) annual solid cattle and liquid hog manure application, short-term (2 years) liquid hog manure application, and short-term (1 year) solid cattle manure application. The solid cattle manure (SCM) and liquid hog manure (LHM) experiments were located approximately 100 m apart in the same field. The long-term experiments were started in the fall of 1996 and short term experiments were started in 2007. In all experiments, the rates of N and P added as manure have been documented along with straw and grain N and P content. Each long- and short-term SCM

and LHM field trial was set up as a randomized complete block design with four replications of each treatment. Manure was applied at low, medium and high rates every year. For each experiment, the low rate of SCM and LHM being applied is approximately 100 kg total N ha^{-1}, and is considered an agronomic rate of manure N that would be recommended to meet a crop N requirement. Medium and high rates of SCM and LHM are considered to be double (200 kg total N ha^{-1}) and quadruple (400 kg total N ha^{-1}) the recommended agronomic rate. These rates are applied annually in October after harvest. The low rate of SCM and LHM in both long and short term experiments was 7.6 T ha^{-1} yr^{-1} (dry-weight) and 37,000 L ha^{-1} yr^{-1}, respectively. The treatment rates and actual rates of P as SCM and LHM made each year based on measurement of concentration of total P in manure from 1997 to 2007 growing seasons are provided in Table 5.1 and total N in manure from 1997 to 2007 growing seasons are provided in Appendix B. The crops grown at the research site from 1997 to 2007 were canola, wheat, barley, canola, wheat, flax, barley, canola, wheat, flax, and oats.

Each experiment had different treatments to address many aspects of manure management, including the effect of application rates, application method, and timing of incorporation (Qian et al., 2004). The long-term SCM and LHM trials consisted of 12 and 15 treatments, respectively. The treatments sampled from both manure trials for this study include control, low, and high rates where SCM was broadcast then incorporated using rototiller and LHM was injected in bands. The short-term SCM and LHM trials consisted of 14 and 10 treatments, respectively. Treatments sampled for this study included the control and the high rate. A detailed description of the long-term field experiment is provided by Stumborg and Schoenau (2008).

Table 5.1. Annual and cumulative amounts of total P in manure applied at Dixon site using solid cattle manure (SCM) and liquid hog manure (LHM) applications.

		Annual total P applied											Total P input
Manure	Treatment	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
		kg P ha^{-1}											
SCM	Low	39	30	20	37	29	60	25	25	18	19	37	339
	High	158	120	80	148	116	240	100	100	72	76	148	1358
LHM	Low	7	3	7	3	3	3	3	6	3	5	19	61
	High	28	12	28	12	12	10	10	25	12	20	76	245

Soil samples were collected from all plots in the spring of 2008 at a depth of 15 cm before crop seeding. Three cores from each plot were collected using polyvinyl chloride (PVC) pipes measuring 15 cm in height and 10 cm in diameter. The cores were removed by excavation, bagged, labeled, removed from the site and stored in a refrigerated facility at 4 ^{o}C for processing. Afterwards, soil was removed from the cores and three cores from each plot were mixed thoroughly to provide a single composite sample. All composite soil samples were then air-dried, ground to pass a 2-mm sieve and stored at room temperature for chemical and XANES analysis.

The selected basic soil characteristics were measured by collecting soil samples from the unfertilized, unmanured control plots. Soil texture was loam and determined using a HORIBA LA-950 Laser diffraction particle-size analyzer (HORIBA Scientific Inc., Edison, NJ, USA). The pH was 7.7 and electrical conductivity (EC) was 0.29 mS cm^{-1}, measured by glass electrode method using a 1:2 soil water suspension (Peters et al., 2003). Organic carbon (OC) was 3.2% determined using the dry combustion method using a LECO-C632 carbon determinator (LECO© Corporation, 1987). Cation exchange capacity (CEC) was 23 cmol kg^{-1} soil measured by 1 *M* NH_4OAc at pH 7.0 (Sumner and Miller, 1996).

5.3.2 Manure sampling and application

The SCM applied in both long- and short-term field trials was obtained from pens in Poundmaker Feedlot, which is located approximately 8 km east of the town of Lanigan, Saskatchewan. The SCM samples were collected by sampling at various points of the feedlot stockpile following the Tri-Provincial manure application and sampling guidelines (Saskatchewan Agriculture and Food, 2003) each year. The LHM applied in both long- and short-term field trials was obtained from a single cell earthen storage unit located approximately 6 km south of the Dixon site at Possberg Pork Farms. The both SCM and LHM were collected

by Prairie Agricultural institute (PAMI) staff each year. Both types of manure collected were immediately frozen and stored at -20 ^{o}C. Before analysis for total P and N, containers were removed from frozen storage and thawed to room temperature. After thawing, sample containers were opened in the laboratory fume hood and stirred to homogenize the contents. Immediately before application, a 5.0 g manure sample was taken and analyzed as follows: Total P and N contents were determined using a standard H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967), followed by colorimetry (Murphy and Riley, 1962). The mean total P concentrations in the manure was used along with the application rate from 1997 to 2007 growing seasons to calculate the total P addition are reported in Table 5.1.

The SCM manure was applied in both long- and short-term field trials into 3.05 by 3.05 m plots each year by hand application, followed by incorporation using a rototiller. The manure from the pens is a mixture of straw bedding material along with cattle feces and urine. The manure was stockpiled for approximately one year prior to application. The LHM was applied in both long- and short-term field plots each year using the PAMI injector truck using coulters to inject manure into 3.0 m by 30.5 m plots to a depth of approximately 10 cm in bands of 30 cm spacing. Both SCM and LHM were applied in the fall (October) each year.

5.3.3 Soil analysis

Labile P was determined using modified Kelowna (MK) (Qian et al., 1994), water extraction (Schoenau and Huang, 1991), and sodium bicarbonate ($NaHCO_3$) extraction (Olsen) methods (Olsen et al., 1954; Tiessen and Moir, 1993). Olsen extractable organic P was determined by subtracting inorganic Olsen P from total Olsen P.

The Kelowna extraction was conducted in all soil samples according to the procedure by Qian et al. (1994). Three grams of soil was weighed into 100 mL plastic containers, and 30 mL

of the MK extractant was dispensed into each of the containers and then shaken horizontally in rotary shaker at 160 rpm for 5 min. The extract was filtered and stored at 4 ºC until samples were analyzed for P colorimetrically using the Murphy and Riley method (1962) on a Technicon Auto analyzer.

The water extractable labile P fraction was determined according to the procedure outlined by Schoenau and Huang (1991) in all soil samples. Two grams of soil from each of the soil samples was placed into plastic containers and 100 mL distilled water was added in each container keeping the soil:water ratio 1:50. The containers were shaken at 200 rpm for 1 h and then decanted through a Millipore^{TM} filter (pore size 0.45 µm) paper. The extractants were then analyzed colorimetrically using the Murphy and Riley method (1962).

The Olsen P fractions were measured by the procedure described by Tiessen and Moir (1993). Briefly a 0.5 g sample of air-dried soil was extracted with 30 mL of 0.5 *M* $NaHCO_3$ (pH 8.5) solution. After extraction, the soil and solution was separated via centrifugation for 10 min at 8,000 x g at 0˚C, and the supernatant was then filtered using a 0.45-µm filter. This extraction process was conducted for 16 h in a rotary shaker for extraction. Total extractable Olsen P fraction was determined using the following procedure. A 10 mL filtered extract was placed into a 50 mL volumetric flask and then 0.5 g of ammonium persulfate and 10 mL of 0.9 *M* H_2SO_4 were added to each flask. The flasks were gently boiled by placing on a hot plate. At this point, about 0.3 mL of solution remained in the flask. Then the flasks were removed from the hot plate and allowed to cool to room temperature. Then 10 mL deionized water was added each flask after cooling. The pH was adjusted using 4 *M* NaOH and 0.25 *M* H_2SO_4 solution and the total P (P_t) was determined using the Murphy Riley method (1962). Another 10 mL aliquent of the filtered extractants was added into a 50 mL centrifuge tube to determine Olsen P_i fractions. The

extractants were acidified to pH 1.5 using 5 mL of 0.9 M H_2SO_4 and stored in a refrigerator for 30 min for precipitation of the organic matter. The extractants were then centrifuged at 8000 × g at 0 ^oC for 10 min, and the extractant was then decanted into 50 mL volumetric flasks. The tubes were rinsed with acidified water, the pH was adjusted to 5.0 to 5.5 if necessary, and P_i was determined colorimetrically according to the method described by Murphy and Riley (1962). The P_o in all of these extracts was calculated by the difference between P_t and P_i in each extract. The absorbance was measured using a spectrophotometer (Beckman DU-65) at wavelength of 712 nm. Soil total P was determined on samples using a standard H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967) followed by colorimetry (Murphy and Riley, 1962) using Technicon Auto analyzer. A brief description of XANES analysis is given below.

5.3.4 X-ray absorption near-edge structure analysis

Phosphorus K-edge XANES spectroscopy was used to determine chemical forms of P in the composite soil samples from the high rate manure treatments. Collection of all P XANES spectra of the soil samples and P standards was conducted at the Soft X-ray Micro-characterization beamline (SXRMB) at the Canadian Light Source (CLS), Saskatoon, Canada. The energy range of this beamline is 1.7-10 keV with a focus beam size of 300µm×300µm. The beamline was calibrated using $ZnPO_4$ powder to the edge energy of 2158 eV in total electron yield (TEY) spectrum and the monochromator is equipped with InSb(III) crystals. The P reference standards, air-dried soil and manure samples were ground into a fine powder and a thin layer was mounted on a stainless steel sample holder using double sided carbon tape. Then the sample holder was placed in the vacuum chamber. The P standards used in this study included brushite ($CaHPO_4.2H_2O$), berlinite ($AlPO_4$), ammonium phosphate monobasic ($NH_4H_2PO_4$), apatite [$Ca_5(PO_4)_3(OH,F,Cl)$], strengite ($FePO_4.2H_2O$), calcium phosphate ($CaHPO_4$),

magnesium phosphate tri-basic [$Mg_3(PO_4)_2$], and phytic acid. A sample of phosphate adsorbed on an Fe oxide (HFO) at pH 6 was synthesized in the lab in a previous study (Peak et al., 2012) and also used as a standard. A spectrum of struvite (($MgNH_4PO_4.6H_2O$) standard used in this study was provided by Dr. Yongfeng, beamline scientist, CLS. The P standards used in this study were purchased as reagent grade powders from chemical manufacturers. The P XANES spectra of these standards are shown in Figure 5.1. The P reference standards spectra were collected in the TEY mode to avoid self-absorption effect, as the self-absorption can distort fluorescence measurements of concentrated samples and standards (Toor et al., 2005; Kar et al., 2011, Peak et al., 2012). Soil and manure sample spectra were collected in partial fluorescence yield (PFY) mode to improve detection limits over total fluorescence or electron yield measurements. The spectra were collected from 40 eV below the absorption edge to 50 eV above the absorption edge (~2110–2200 eV). The step size in the pre edge region (2110-2145 eV) was 1 eV, 0.25 eV in near edge region (2145.25-2180 eV), and 0.5 eV in post edge region (2180.5-2200 eV) with a constant dwell time of 4.0 s.

All the P XANES spectra analyses were performed using WinXAS version 3.1 software. Multiple scans were collected for each sample. The multiple spectra were averaged to obtain an adequate signal to noise ratio for spectral analysis. The background of averaged XANES spectra was corrected using a first-order polynomial fit to the pre-edge region from 2,135 to 2,145 eV. The spectra were then normalized to an edge jump of 1.0 over the region from 2,140 to 2,180 eV. Linear combination (LC) fitting of soil and manure XANES spectra was conducted over the spectral region from 2,140 to 2,180 eV. This large energy range was used to include more chemical information and therefore increase the probability that similar standards can be properly distinguished (Toor et al., 2005; Peak et al., 2012). During the LC fitting, the initial

threshold energy, E_0 value was allowed to vary to provide an initial estimate of percent contribution, but in the second iteration E_0 values were fixed to ‘‘0’’ for all components since the beamline was calibrated using a $ZnPO_4$ standard that was scanned regularly during data collection.

5.3.5 Statistical analysis

Data from wet chemical analysis were statistically analyzed using the data analysis package in Microsoft Excel 2010 (Microsoft, Seattle, WA) to calculate mean and standard deviation. The significance of treatment effects was determined at 5% probability level and was attained by student t-test for short-term study and one way ANOVA for long-term study data using Statistical Package of Social Science (SPSS Inc.).

5.4 Results and Discussion

5.4.1 Short and long-term manure addition effects on soil total and labile phosphorus

The soil total and labile P concentrations measured by different STP methods from short- and long-term SCM and LHM amended soils are shown in Table 5.2. Both soil total P and STP concentrations increased with addition of both manure types and with increasing application rates. Effects were greatest and most often significant in the long-term SCM experiment. Similar to our results, Chang et al. (2005) conducted a repeated manure application experiment in southern Alberta and reported that soil total P and STP concentrations increased with the manure addition regardless of application rate. In the current study, only the long-term SCM high rate resulted in a statistically significant increase in total P content (Table 5.2). Long-term 30 year continuous application of cattle manure has been reported to lead to the accumulation of P in soils (Hao et al., 2008). Our results showed that short-term (1 year) and the low rate (8 Mg ha^{-1} dry wt.) of SCM application for 11 years did not lead to measurable significant increase in total P

Table 5.2. Effect of short-term (1-2 year) and long-term (11 years) animal manure applications on soil total P and labile P concentrations.

Treatment	Total-P	H_2O-P	KM-P	$NaHCO_3$-P_i	$NaHCO_3$-P_o
	mg P kg^{-1}				
			Short-term		
SCM†					
Control	634a§	10 (2)‡a	16(3)b	74(11)a	28(4)a
High rate	734a	22(3)b	49(7)a	76(10)a	37(5)a
LHM††					
Control	845a	9(1)a	11(1)b	56(7)a	23(3)a
High rate	876a	13(1)a	20(2)a	83(9)a	31(4)a
			Long-term		
SCM					
Control	743b	8(1)c	18(2)c	56(5)c	16(2)c
Low rate	871b	37(4)b	81(9)b	153(18)b	69(8)b
High rate	1108a	87(8)a	224(20)a	416(38)a	111(10)a
LHM					
Control	613a	6(1)b	7(1)b	29(5)b	15(2)a
Low rate	635a	7(1)b	9(1)b	34(5)b	18(3)a
High rate	685a	13(2)a	26(4)a	74(11)a	19(3)a

†SCM, solid cattle manure; ††LHM, liquid hog manure

‡ The numbers in parentheses are the fraction as a percentage of total P in each treatment

§Means (n = 4) in a column for each manure followed by different letters are significantly different at $P \leq 0.05$. Analysis was performed using Student t test and Tukey's test using one way ANOVA for short-term and long-term study, respectively.

in the surface soil. Higher soil total P content with long-term SCM application agrees with Stumborg and Schoenau (2008), who similarly reported that soil total P concentration was higher after 8 years and that it increased with increasing application rate (7.6-30.4 Mg ha^{-1} yr^{-1}), as manure P input exceeds crop removal of soil P. Parham et al. (2002) has shown that soil P increased, ranging from 4.5 to 10.3 kg P ha^{-1} yr^{-1} compared with the control soil, in soils from a long-term (64 years) cattle manure experiment in Oklahoma where the annual manure application rate was 22.4 kg P ha^{-1} yr^{-1}.

The LHM additions over short (2 years) or long term (11 years) did not result in statistically significant increases in soil total P (Table 5.2). Qian and Schoenau (2000a) conducted a short-term experiment in a Black Chernozemic Saskatchewan soil and reported that a single application of swine manure at low and high rates did not have a significant impact on total soil P because of lower amounts of total P in swine manure compared to cattle manure. The repeated addition of LHM stimulated plant growth in response to added N (Mooleki et al., 2002), and increased the crop removal of P without adding a large amount of P due to the wide N:P ratio of the manure used. Manure with high content and availability of N can provide a well-balanced supply of nutrients and can increase plant P uptake and removal (Qian and Schoenau 2000b). After 5 and 8 years of manure application at this same site, the low and medium rate of LHM addition had higher removal of P in grain harvest than the amount of P added (Qian et al., 2004; Stumborg and Schoenau, 2008). The highest rate of LHM would provide approximately 15 times less P than highest rate of SCM at this site (Stumborg and Schoenau, 2008), and relatively small P deficits or surplus in the treatments are consistent with the lack of a significant impact of LHM on total P concentration.

The amount of labile P extracted by different STP methods follows the order Olsen extractable > KM > water extractable in both short- and long-term manure amended soils (Table 5.2). The STP methods used in this study were previously shown to be significantly correlated with each other (Stumborg and Schoenau, 2008; Kashem et al., 2004) which indicate that any one of these methods can be used to assess labile P in calcareous soils. However, the bicarbonate extractable P was less effective in revealing significant differences, especially in the short-term manured soils. It was suggested that water extraction was less sensitive to the effects of manure P loading compared to using chemical extractants (Wright et al., 2003), but gives a measurement

of relative P availability independent of soil type (Schoenau and Huang, 1991). Water extracted the least amount of P from both short- and long-term manured and unmanured soils, but was sensitive to manure P addition in both short- and long-term. The other STP methods extract P from soil using chemical extracting solution which, unlike water extraction, is based on alteration of pH and use of ions to desorb and dissolve solid phase P.

In soil amended with SCM for one year, the labile P concentrations were significantly increased over control with the exception of $NaHCO_3$ extraction (Table 5.2). A significant increase of P extracted by all STP methods was observed after long-term (11 annual) additions of SCM. Overall, differences among treatments were greater after long-term than short-term additions and this difference increased with increasing application rate. The $NaHCO_3$ fractions appear to be a significant reservoir for SCM P added over a number of years, as the $NaHCO_3$ P_i comprised 38% of total P at the high rate in the long-term and 10% of total P in the short-term high rate. Kashem et al. (2004) reported that greater amounts of labile P fractions were extracted from soils amended with cattle manure compared to hog manure applications, but the labile P fraction increased with the manure application rates for both types of manure. Large amounts of manure P accumulating in the $NaHCO_3$ Pi fraction over several years suggests that added P from SCM may be entering into surface adsorbed and precipitated forms. Several researchers (MnKeni and MacKenzie, 1985; Domaar and Chang, 1995; Tran and N'dayegamiye, 1995) have reported that increased accumulation of manure P in labile P_i fractions after long-term manure application could be the cumulative effect of manure additions, coupled with a decrease in the soil P adsorption capacity as fixation sites become saturated (MnKeni and McKenzie, 1985). Laboski and Lamb (2003) reported enhanced availability of P in manure-amended calcareous soils, and attributed it to organic acids in the manure that prevented P precipitation and reduced

the rate of formation of more crystalline and less soluble P minerals. The SCM used in this study has a larger organic P component than LHM because SCM contains a significant amount of organic P from feed passing through the animal and due to the mixing of straw bedding materials during production (Ajiboye et al., 2004). The straw can stimulate immobilization processes that lower nutrient availability and plant uptake. In SCM, the initial high concentration of P immobilized in organic matter results in lower P plant availability initially, but contributes to increasing labile STP concentrations as mineralization processes occur with time.

For the LHM amended soil, the short-term manure (two years) application did not have strong impact on P availability in the soil (Table 5.2). In general, there was no significant effect of LHM application on labile P in the short-term. Only Kelowna extractable P (2% of total P) showed a small but significant increase over the control. The long-term LHM additions revealed that labile P increased with increasing application rate, but significant increases of labile P only occurred at the high rate (11% of total P) and the increase was not as great as that found with SCM. Findings from the short-term study conducted by Qian and Schoenau (2000a) was similar to these results, where a single application of swine manure at low and high rates did not have a significant impact on increasing labile soil P because of lower amounts of available and total P in swine manure compared to cattle manure. Stumborg and Schoenau (2008) reported similar results in a field study after 8 years of LHM additions with same soil. The P input from LHM is lower than SCM (Table 5.1) and the greater amount of available nitrogen in LHM stimulated enhance plant growth and increased plant P uptake and crop removal.

5.4.2 Solid-state soil phosphorus speciation via XANES analysis

The spectra of the different standard phosphate compounds used in LC fitting for this study are shown in Figure 5.1. The different standard phosphate compounds have characteristic

features in main peak position as well as in number and position of pre-edge and post-edge peaks that can be used to identify phosphate species in the XANES spectra of the amended soils. The spectra are normalized and offset for comparison. In all spectra, the main white line energy occurred near 2154 eV due to 1s→3p electron transition and a broader peak occurred at around 2169 eV due to oxygen oscillation (Kruse and Leinweber, 2008). The XANES spectra of mono-ammonium phosphate and phytic acid were characterized by intense white line peak and short and broader white line peak, respectively. Spectra for Fe(III)-phosphate (strengite) and Al(III)-phosphate (berlinite) were distinctive from each other; Ferric-phosphate showed distinguishing pre-edge feature at around 2148 eV and Al-phosphate were characterized by very weak pre-edge inflection at about 2152 eV and a shorter white line peak. All calcium phosphate minerals showed the same characteristic spectral feature on the high energy side of the main white line peak between 2156 and 2166 eV. Some researchers (Hesterberg et al., 1999; Sato et al., 2005; Khatiwada, 2011) have reported that this feature is more defined for calcium phosphate minerals with decreasing solubility; both the stability of a calcium phosphate phase and increasing crystallinity within a phase increase this feature. For example, in our study, apatite showed a more pronounced shoulder on the high energy side of the main white line peak compared to brushite. Spectra of Mg-phosphate minerals showed the broad spectral feature at around 2160 eV that was more pronounced in struvite ($MgNH_4PO_4.6H_2O$) as compared to newberyite [$Mg_3(PO_4)_2$]. The adsorbed P species usually had more intense and narrow main white line peak. These spectral features of different phosphate standard materials have been reported by many researchers (Hesterberg et al., 1999; Peak et al., 2002; Beauchemin et al., 2003; Khare et al., 2005; Sato et al., 2005; Lombi et al., 2006; Güngör et al., 2007; Kruse and Leinweber, 2008) and

can be used to differentiate the association of phosphate with different elements in soil samples amended with organic or inorganic P fertilizer.

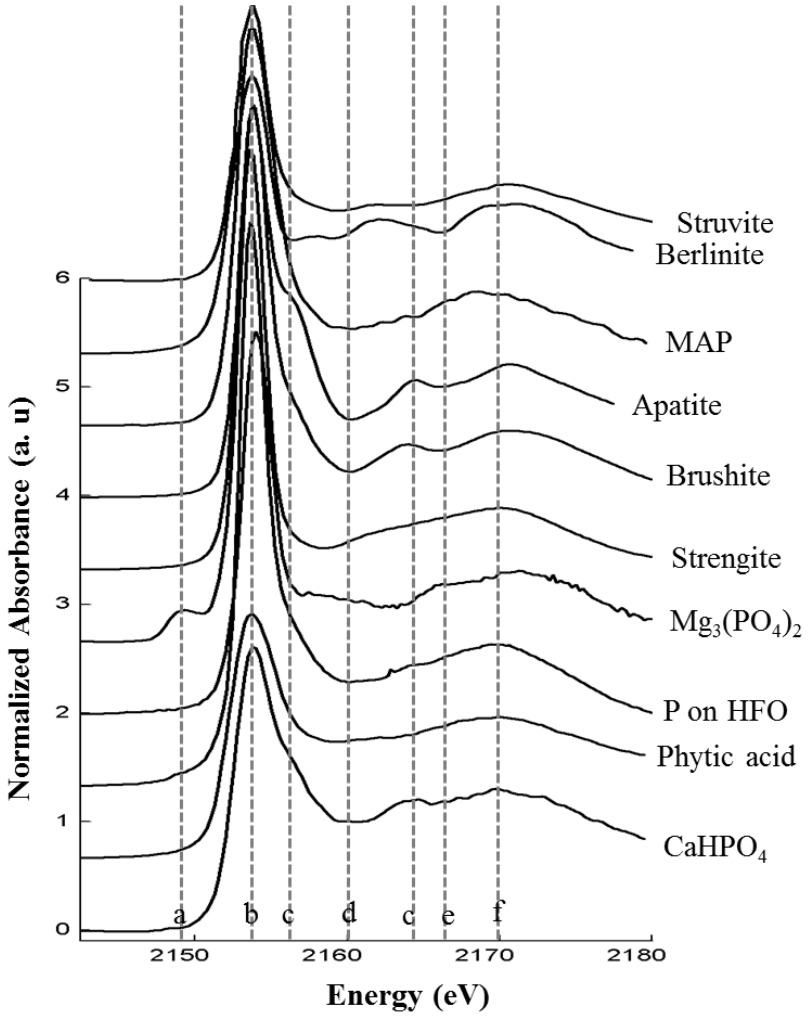

Figure 5.1. Normalized P K-edge X-ray absorption near edge structure spectra of different P standards: struvite ($MgNH_4PO_4.6H_2O$), berlinite ($AlPO_4$), MAP ($NH_4H_2PO_4$), apatite [$Ca_5(PO_4)_3(OH,F,Cl)$], brushite ($CaHPO_4.2H_2O$), strengite ($FePO_4.2H_2O$), Mg phosphate tribasic ($Mg_3(PO_4)_2$), phosphate adsorbed on an Fe oxide (P on HFO), phytic acid, and $CaHPO_4$ used in linear combination fitting. Spectra are offset by 0.6 absorbance units to allow for comparison of spectral features. The dashed lines show unique spectral features for different P minerals: the pre-edge of (a) Fe phosphate, (b) white line peak, (c) spectral features of Ca phosphate, (d, e) spectral features of Al and NH_4 phosphates, and (f) oxygen oscillation.

The LC fitting results of XANES spectra of the dominant P species in the manure amended soils are shown in Figure 5.2. The fitting results of manure amended soils showed that

more soluble Ca phosphate species such as brushite was the dominant P species in SCM amended soil while adsorbed-P dominated the P speciation in LHM amended soil.

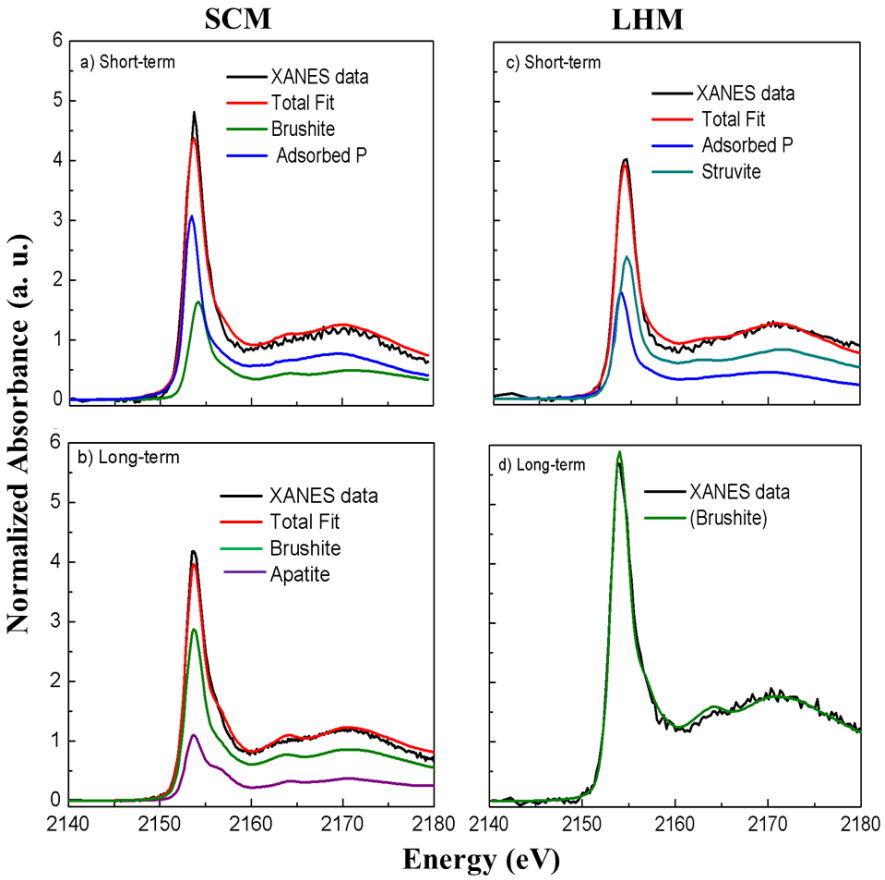

Figure 5.2. Linear combination XANES fit of differential XANES spectra of solid cattle manure (SCM) short-term (a) and long-term (b) and liquid hog manure (LHM) short-term (c) and long-term (d) amended soils.

The LC fits of SCM applied soil (Figure 5.2a, Table 5.3) revealed that the short-term SCM treated soil was dominated by adsorbed P (62%) and brushite (38%), consistent with observations made in the banded SCM soil reported on in Chapter 4, while the majority of P was present as brushite (69%) and the rest was accounted for as less soluble Ca phosphates like apatite (31%) in long-term SCM treated soil. The preponderance of brushite in the long-term SCM soil is consistent with the accumulation of P in the $NaHCO_3$ fraction, suggesting that

brushite may be the main component of $NaHCO_3$-P_i. Similar to our results, Ajiboye et al. (2008) also reported that a high proportion of P was present as adsorbed form in short-term SCM manure amended soil. Repeated application of SCM over several years appears to have resulted in the transformation of adsorbed P and/or accumulation of P in relatively soluble (brushite) and also less soluble calcium phosphate minerals (apatite). Consistent with this, Ca:P ratio and soil pH were both lower in the short-term SCM soil compared to long term. The pH increases after long term cattle manure addition was also reported by Parham et al. (2002) in Oklahoma in the USA. This is also supported by our wet chemical analysis where water and $NaHCO_3$ extractable P were much higher after long-term SCM application. The presence of high proportion of relatively soluble calcium phosphate mineral (brushite) after long-term SCM addition could be due to presence of organic acids in SCM that can inhibit formation of thermodynamically stable Ca-P minerals (Grossl and Inskeep, 1992) by blocking soil P adsorption sites (Kashem et al., 2004).

Table 5.3. Linear combination phosphorus X-ray absorption near edge structure (P XANES) fitting results showing proportions of P compounds in soils after short- and long-term solid cattle manure and liquid hog manure additions.

Treatment	pH	Ca:P ratios	Brushite	Adsorbed P	Apatite	Struvite
				%		
ST-SCM	7.2	2.5	38	62	-	-
LT-SCM	7.9	3.2	69	-	31	-
ST-LHM	7.8	2.3	-	27	-	73
LT-LHM	7.3	2.1	100	-	-	-

SCM, solid cattle manure; LHM, liquid hog manure
ST, short term; LT, long term

For the LHM amended soil, it was shown by the fitting results (Figure 5.2c, Table 5.3) that the short-term LHM amended soil was dominated by struvite and adsorbed P species, which were 73% and 27%, respectively. On the other hand, P was only observed as brushite form in

long-term LHM amended soil (Figure 5.2d, Table 5.3). The likely explanation for this is that decreased pH and lower Ca:P ratio after long-term application could favour the desorption/dissolution of Mg phosphate (struvite). The LHM contained higher amount of available N as ammonium (~70% of total N) compared to SCM and pH decreased over time as a result of acidification produced from nitrification of the ammonium (~75% of total N) originating from LHM and dissolution of struvite (NH_4MgPO_4). This decreased pH would enhance P sorption on variably charged soil surfaces (edges of clay minerals and iron oxides). Overall, the dominance of brushite in SCM treated soils and adsorbed P in LHM treated soils implies that the initial mechanisms for P retention seems to be mainly precipitation in SCM treated soils and adsorption in LHM treated soils. Organic P species in manured soil samples were not identified by XANES spectroscopy. Several researchers (Beauchemin et al., 2003; Sato et al., 2005; Ajiboye et al., 2008) have reported that organic P as phytic acid was not detected by XANES in bulk soils; it has been reported that this may be due to difficulty in identification of organic P using K-edge XANES.

5.5 Conclusions

This study evaluates the effect of short and long-term cattle and hog manure applications on soil P forms and distribution in a calcareous Canadian prairie soil. Manure application increased the total and labile P levels in soil with increasing years of application. The magnitude of the increase was much higher in SCM application than LHM application. The long-term SCM application significantly increased the labile P fractions with significant amounts of P residing in the $NaHCO_3$ fraction after 11 years of SCM application. Linear combination fitting of XANES spectra showed that short-term application of SCM to soil resulted in phosphates released and identified as adsorbed P and soluble Ca-P minerals (brushite). With long-term repeated

application of SCM, Ca-P minerals dominated as adsorbed P appeared to be transformed to soluble (brushite) and more stable (apatite) Ca-P minerals in soil. In short-term LHM amended soil, struvite was the dominant phosphate mineral, with small amounts of adsorbed P which indicates that precipitation was the main retention mechanism in this soil. Over time, repeated LHM application resulted in the apparent transformation of struvite into brushite presumably via dissolution of orthophosphate from Mg phosphate (struvite). The wet chemical analysis and XANES results showed that long term annual application of cattle manure to Saskatchewan soils has pronounced effects on total and labile P even at the recommended low rate of application. This effect is even more pronounced with long-term application rates. These results indicate that P from SCM in excess of crop requirements will lead to a large accumulation of STP in soil, which poses a potential risk of P losses from agricultural soil through runoff and erosion.

CHAPTER 6.0

SYNTHESIS AND CONCLUSIONS

Manure contains significant amounts of P that can contribute to plant available, labile P when added to soil. However, large pools of soil P that are soluble can also contribute to P export off-site in runoff and leaching water. The fate of added P as manure and fertilizer in the soil in terms of its residence in different chemical forms and their transformations over time is important to know. Improving the fundamental knowledge of fate and transformation of P species in calcareous prairie soils, specifically those amended with either manure or inorganic fertilizer over widely different time frames was the objective of this dissertation. In the past, researchers have used many laboratory-based wet chemical analysis methods to quantify total P and separate P into different inorganic and organic fractions, but it was not clear what chemical forms of P were contained in these fractions. Forms of P and the transformation processes they undergo in soil are affected by soil conditions and different amendment management practices. These effects and the changes that occur over different time scales (weeks, months, years) following addition are useful to know. To help address these questions effectively, advanced molecular-based synchrotron techniques can potentially provide a wealth of useful information related to P chemistry in soils. In this dissertation, advanced spectroscopic techniques were successfully combined with wet chemical analysis techniques to answer questions related to the soil chemistry of applied P. Prior to this study, it was unclear whether X-ray absorption near-edge structure spectroscopy would be able to speciate P at the low P contents normally found in agricultural soils under field conditions. Very little work has been done using synchrotron techniques applied to soil receiving typical rates of P application, especially in prairie soils such

as those in Saskatchewan. This study was successful in identifying P forms, amounts, and distribution of P minerals under such conditions.

6.1 Research Findings

Three studies were conceived and structured to address the forms, nature, and transformations of P in amended soils from the molecular scale to field scale over a time frame starting from very short-term (days and weeks) to short-term (months to a year) periods, along with assessment of the effects of long-term (11 years) repeated annual applications. It was observed that fate and transformation of applied P is strongly affected by fertilizer type, plant growth, landscape position and soil conditions, rate, placement, and duration of application.

The most labile (resin-P and $NaHCO_3$-P_i) and more stable (Ca-associated) P forms were depleted in the presence of plants in solid cattle manure amended and unamended control soils over a period of five weeks following application. In contrast, moderately labile inorganic P forms extracted by NaOH increased with time in all cases, suggesting a replenishment of these forms through mineralization of moderately labile organic P (NaOH-P_o) or solubilization of more stable Ca-P minerals. The XANES analysis did not show any detectable changes in bulk speciation or crystallinity of P after 5 weeks of plant growth. Therefore P K-edge XANES has some limitations; it is challenging to identify adsorbed, aqueous, organic, and poorly crystalline Ca-P due to lack of distinct spectral features of these minerals, and to detect small changes in relative abundance of different P forms that may be occurring over the very short term. Overall, the results indicate that the initial redistribution of P upon mixing manure into a soil is the dominant chemical effect that generally persists for the first few weeks following application. Plant growth in this period is effective in reducing concentrations of labile, potentially mobile P forms.

Soil properties typically vary across landscape positions in a field. Assessment of treatment effects in contrasting slope positions can reveal important soil property-nutrient fate relationships. The upslope positions in a typical hummocky southern Saskatchewan farm field had pH > 7 while downslope position had pH < 7, along with high $CaCO_3$ content in upslope positions and lower organic matter (OM) content than downslopes. It was observed that one month following application of manure and inorganic fertilizer in a band across the landscape of two farm fields, the form the P was found to reside in was controlled by several factors related to landscape position including pH, OM content, Ca content, presence of Al and Fe oxides, as well as distance from application zone. A similar pattern of labile P supply and total P concentrations were observed in both manure and inorganic fertilizer application bands in both slope positions. Application of fertilizer P in a band increased the total and labile P supply at the center of the band regardless of slope position. The labile P supply rate in the band center was more than double that of the soil 10 cm away from the band center, confirming that the influence of the band fertilizer P was confined to a relatively small area. This is consistent with the accepted view that P movement from the point of application is limited to about 3 to 5 cm. The release of P from OM over time and physical movement of labile P through runoff and erosion from upslope increased total and labile P supply in the downslope position.

The XANES results coupled with chemical extractions showed that different P transformation mechanisms occur, depending upon both landscape position and amendment type. The transformation process was apparently controlled by several factors such as pH, Ca:P ratio, OM content. The higher pH, higher Ca:P ratio, and low OM content found in upslope positions favoured enhanced formation of less soluble apatite-like Ca-P minerals and the abundance of these minerals increased as the Ca:P ratio increased. In contrast, the lower pH and presence of

more OM in the downslope position likely prevented the formation of more stable Ca-P minerals via a combination of complexation of soluble Ca ions by organic ligands, adsorption of P on metal (Al, Fe) oxides, and an increase in the stability of more labile Ca phosphates (brushite) with a smaller Ca:P ratio. The center of the band of inorganic fertilized upslope soil in the Brown soil zone was dominated by adsorbed P species along with apatite, while the manured upslope soil in the Black soil zone was dominated by DCP. The downslope position was dominated by adsorbed P species at all distances from the band center. The finding from both wet chemical analysis data and XANES results of this study indicate that the applied P was mainly present as labile and moderately labile forms in the center of the band one month after application. It was also observed that the manure amended band center contained localization concentration of organic P in the center of the band that likely was derived from the manure itself rather than formed in the soil.

The soil P forms and distribution after short- (1-2 years) and long-term (11 years) annual applications of cattle and hog manure are described in Chapter 5. Long-term SCM addition significantly increases the total P concentration in soil due to repeated P addition in excess of crop requirement. The SCM application also resulted in significantly higher levels of labile P concentration regardless of application rate compared to LHM and control soils. The magnitude was much higher after long-term application. This is attributed to high concentration of P in SCM compared to LHM and continued contribution to labile P through mineralization of organic P over time.

With LHM, a smaller increase of labile P fraction was observed, and only after long-term application at the high rate. This is due to the lower amount of P added with the LHM than the SCM. P speciation in SCM amended soil revealed mainly adsorbed P and soluble Ca-P minerals

(brushite) forms after short-term application. Over time, repeated application resulted in prevalence of brushite along with formation of more stable apatite-like Ca-P minerals. This transformation of adsorbed P and more soluble (brushite) into less soluble Ca-P minerals (apatite) may be caused by a combination of factors including high pH and increasing Ca:P ratio over time. Another important finding is the dominance of brushite in the long-term SCM amended soil which agrees with the wet chemical analysis where a large amount of P was extracted by $NaHCO_3$ extraction, and indicates that brushite may be the main component of $NaHCO_3$ P_i. This result confirms that P XANES can provide further insight into the precise chemical nature of P species in amended soils if combined with wet chemical analysis data.

Phosphate in short-term LHM amended soil was present mainly as struvite and a small amount as soluble Ca-P minerals such as brushite and over a longer time (years) it appeared to be present mainly as poorly crystalline brushite forms. Over time, repeated LHM application resulted in the apparent transformation of struvite into brushite form presumably via dissolution of orthophosphate from Mg-phosphate (struvite). No changes in organic P species were identified by XANES spectroscopy, which is a limitation of XANES; this was also observed in previous studies and reported by many other researchers. The combined findings from wet chemical analysis and XANES spectroscopy of this study indicate that the repeated P additions from SCM in excess of crop removal significantly elevated total and labile P, and subsequently would increase the potential of P transfer from agricultural soils to waterways through leaching and runoff. The application of LHM based on crop N requirements poses lesser issue for P accumulation and loss.

6.2 Implications and Recommendations for Future Research

The implication of the findings from the study of soil P forms over the very short term

(1 to 5 weeks) following manure application is that the enhancement of labile, bioavailable, and mobile forms arises in the period shortly following application, and that for some manure forms like SCM, some replenishment occurs from more stable inorganic P forms when labile P is depleted via plant uptake. A rising soil content of labile P forms may be mitigated by the presence of growing plants that utilize labile P. This work was performed under controlled conditions; the next step would be to examine the effects of manure in presence or absence of plants under field conditions.

The preponderance of labile P forms found at the center of fertilizer bands after short-term application supports banding as an appropriate application strategy for increasing P availability, especially in calcareous soils. The development of a novel sampling technique (Chapter 4) for spectroscopic manure P speciation at low P contents is an advance that will greatly expand the utility of soft x-ray techniques in soil and agronomic research. Further studies are needed to investigate the role that these speciation changes may have on plant uptake, to determine if the retention mechanisms for inorganic fertilizer and manure P change over longer time scales and with repeated applications.

The P from SCM in excess of crop requirements applied over several years will lead to a large accumulation of labile and adsorbed P in soil, which poses a potential threat of P losses from agricultural soil through runoff and erosion. Thus, the application of SCM based on crop P requirement with addition of supplemental fertilizer N, or application every consecutive 2nd year and maintaining high soil pH is recommended, since brushite and apatite are least soluble at high pH. The apparent impacts of manure type and application history noted in this study deserve further attention by including a wider variety of manure and soil types, and longer application histories.

Overall, the application of XANES spectroscopy in this dissertation was limited to P K-edge measurements. A major limitation of this technique is a lack of sensitivity to detect organic P species, arising from a lack of distinct features in the P K-edge XANES of those compounds. The near edge spectra of other electronic transitions and decay processes involved with other core level electrons apart from 1s (K-edge) need to be investigated for organic P compounds in soil. For example, P L-edge XANES spectra of organic P compounds may be a significant improvement of this technique and should be developed for soils.

CHAPTER 7.0

REFERENCES

Achkar, A.J., T.Z. Regier, H. Wadati, Y.J. Kim, H. Zhang, and D.G. Hawthorn. 2011. Bulk sensitive x-ray absorption spectroscopy free of self-absorption effects. Phys. Rev. 83:81106-81109.

Adams, M.A., and L.T. Byrne. 1989. ^{31}P-NMR analysis of phosphorus compounds in extracts of surface soils from selected karri (*Eucalyptus diversicolor* F. Muell) forests. Soil Biol. Biochem. 21:523-528.

Adeli, A., K.R. Sistani, M.F. Bal'a, and D.E. Rowe. 2005. Phosphorus dynamics in broiler litter amended soils. Commun. Soil Sci. Plant Anal. 36:1099-1115.

Ajiboye, B. 2007. Molecular speciation of phosphorus in organic amendments and amended soils using nuclear magnetic resonance and X-ray absorption spectroscopies. Ph.D. diss. University of Manitoba, Winnipeg, MB, Canada.

Ajiboye, B., O.O. Akinremi, and G.J. Racz. 2004. Laboratory characterization of P in fresh and oven-dried organic amendments. J. Environ. Qual. 33:1062-1069.

Ajiboye, B., O.O. Akinremi, Y. Hu, and A. Jürgensen. 2008. XANES speciation of phosphorus in organically amended and fertilized vertisol and mollisol. Soil Sci. Soc. Am. J. 72: 1256-1262.

Akhtar, M., D.L. McCallister, D.D. Francis, and K.S. Schepers. 2005. Manure source effects on soil phosphorus fractions and their distribution. Soil Sci. 170:183-190.

Amer F., D. Bouldin, C.A. Black, and F.R. Duke. 1955. Characterization of soil phosphorus by an anion exchange resin adsorption and ^{32}P equilibrium. Plant Soil 6:381-409.

Armstrong, R.D., and K.R. Helyar. 1992. Changes in soil phosphate fractions in the rhizosphere of semi-arid pasture grasses. Aust. J. Soil Res. 30:131-143

Atia, A.M., and A.P. Mallarino. 2002. Agronomic and environmental soil phosphorus testing in soils receiving liquid swine manure. Soil Sci. Soc. Am. J. 66:1696-1705.

Baldwin, C.S. 1983. A barnyard story. Soils Section. Ridgetown College of Agric. Tech. Ridgetown, Ontario. Bull. NOP.

Barnett, G.M. 1994. Manure P fractionation. Bioresour. Technol. 49:149–157.

Barrow, N.J. 1960. The effect of varying the nitrogen, sulfur, and phosphorus content of organic matter of its decomposition. Aust. J. Agric. Res. 11:317-330.

Beauchamp, E.G. 1987. Corn response to residual N from urea and manures applied in previous years. Can. J. Soil Sci. 67:931-942.

Beauchemin, S., D. Hesterberg, J. Chou, M. Beauchemin, R.R. Simard, and D.E. Sayers. 2003. Speciation of phosphorus in phosphorus-enriched agricultural soils using X-ray absorption near-edge spectroscopy and chemical fractionation. J. Environ. Qual. 32:1809-1819.

Beck, M.A., and P.A. Sanchez. 1994. Soil phosphorus fraction dynamics during 18 years of cultivation on a Typic Paleudult. Soil Sci. Soc. Am. J. 58:1424-1431.

Beegle, D.B. 2005. Assessing soil phosphorus for crop production. Pp. 123–143 *in* J.T. Sims and A.N. Sharpley, (eds) Phosphorus: Agriculture and the environment. Agronomy Monograph No. 46. ASA, CSSA, SSSA, Madison, WI.

Bertrand, I., P. Hinsinger, B. Jaillard, and J.C. Arvieu. 1999. Dynamics of phosphorus in the rhizosphere of maize and rape grown on synthetic, phosphated calcite and geothite. Plant Soil. 211:111-119.

Bertrand, I., R.E. Holloway, R.D. Armstrong, and M.J. McLaughlin. 2003. Chemical characteristics of phosphorus in alkaline soils from southern Australia. Aust. J. Soil Res. 41:61-76.

Blanchar, R.W., and A.C. Caldwell. 1966. Phosphate-ammonium-moisture relationships in soils. I. Ion concentrations in leached fertilizer zones and effects on plants. Soil Sci. Soc. Am. Proc. 30:39-43.

Bowman, R.A. 1988. A rapid method to determine total phosphorus in soils. Soil Sci. Soc. Am. J. 52:1301-1304.

Bowman, R.A., and C.V. Cole. 1978. An exploratory method for fractionation of organic phosphorus from grassland soil. Soil Sci. 125:95-101.

Brady, N.C., and R.R. Well. 2002. The nature and properties of soil. Pearson Education, Inc., NJ.

Brandt, R.C., H.A. Elliott, and G.A. O'Connor. 2004. Water extractable phosphorus in biosolids: Implications for land-based recycling. Water Environ. Res. 76:121-129.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus is soils. Soil Sci. 59:39-45.

Bremner, J.M., and C.S. Mulvaney. 1982. Total nitrogen. pp. 595-624. *In* A.L. Page et al.(ed) Methods of soil analysis. Vol. 2. 2nd ed. Agron. Monogr. 9. ASA, Madison, WI.

Bril, J., and W. Salomons. 1990. Chemical-composition of animal manure-A modeling approach. Neth. J. Agric. Sci. 38:333-351.

Buehler, S., A. Oberson, I.M. Rao, D.K. Friesen, and E. Frossard. 2002. Sequential phosphorus extraction of a ^{33}P-labeled Oxisol under contrasting agricultural systems. Soil Sci. Soc. Am. J. 66:868-877.

Campbell, C.A., M. Schnitzer, J.W.B. Stewart, V.O. Biederbeck, and F. Selles. 1986. Effect of manure and P fertilizer on properties of a Black Chernozem in southern Saskatchewan. Can. J. Soil Sci. 66:601-613.

Canadian Light Source Inc. 2007. [Online] Available at:

Chang, C., J.K. Whalen, and X. Hao. 2005. Increase in phosphorus concentration of a clay loam surface soil receiving repeated annual feedlot cattle manure applications in southern Alberta. Can. J. Soil Sci. 85:589-597.

Chang, S.C., and M.L. Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133-144.

Chardon, W.J., O. Oenema, P. del Castilho, R. Vriesema, J. Japenga, and D. Blaauw. 1997. Organic phosphorus in solutions and leachates from soils treated with animal slurries. J. Environ. Qual. 26:372-378.

Charles, J.L. 1999. Soil and crop response to hog and cattle manure additions in east central Saskatchewan, MS thesis, University of Saskatchewan, Saskatoon, SK.

Chase, C., M. Duffy, and W. Lotz. 1991. Economic impact of varying swine manure application rates on continuous corn. J. Soil Water Conserv. 46:460-464.

Condron, L.M., and K.M. Goh. 1989. Effects of long-term phosphatic fertilizers applications on amounts and forms of phosphorus in soils under irrigated pasture in New Zealand. J. Soil Sci. 40:383-395.

Condron, L.M., E. Frossard, R.H. Newman, P. Tekely, and J.L. Morel. 1997. Use of ^{31}P NMR in the study of soils and environment. *In* "Nuclear magnetic resonance spectroscopy in environmental chemistry" (J.A. Leenheer, Ed.). Oxford University Press, New York, Oxford.

Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 28:261-266.

Daniel, T.C., A.N. Sharpley, D.R. Edwards, R. Wedepohl, and J.L. Lemunyon. 1994. Minimizing surface water eutrophication from agriculture by phosphorus management. J. Soil Water Conserv. 49:30-38.

Dick, W.A., and M.A. Tabatabai. 1977. An alkaline oxidation method for determination of total phosphorus in soils. Soil Sci. Soc. Am. J. 41:511-514.

Djodjic, F., K. Borling, and L. Bergstrom. 2003. Phosphorus leaching in relation to soil type and soil phosphorus content. J. Environ. Qual. 33:678-684.

Dormaar, J.F., and C. Chang. 1995. Effects of 20 annual applications of excess feedlot manure on labile soil phosphorus. Can. J. Soil Sci. 75:507-512.

Dou, Z., J.D. Toth, D.T. Galligan, C.F. Ramberg, Jr., and J.D. Ferguson. 2000. Laboratory procedures for characterizing manure phosphorus. J. Environ. Qual. 29: 508-514.

Dou, Z.X., K.F. Knowlton, R.A. Kohn, Z.G. Wu, L.D. Satter, G.Y. Zhang, J.D. Toth, and J.D. Ferguson. 2002. Phosphorus characteristics of dairy feces affected by diets. J. Environ. Qual. 31:2058-2065.

Douglas, C.L., Jr., and S.L. Albrecht. 2000. Burn or bale: effect on biomass and nutrients. Pages 46-50 in Columbia Basin Agricultural Research Annual Report. Spec. Rpt. 1012. Oregon State Univ. and USDA-ARS, Pendleton, OR.

Eghball, B. 2003. Leaching of phosphorus fractions following manure or compost application. Commun. Soil Sci. Plant Anal. 34:2803-2815.

Eghball, B., and D.H. Sander. 1989. Distance and distribution effects of phosphorus fertilizer on corn. Soil Sci. Soc. Am. J. 53:282-287.

Eghball, B., and J.F. Power. 1999. Phosphorus and nitrogen based manure and compost applications: corn production and soil phosphorus. Soil Sci. Soc. Am. J. 63:895-901.

Eghball, B., D.H. Sander, and J. Skopp. 1990. Diffusion, adsorption, and predicted longevity of banded phosphorus fertilizer in three soils. Soil Sci. Soc. Am. J. 54:1161-1165.

Elder, F.R., A.M. Gurewitsch, R.V. Langmuir, and H.C. Pollock. 1947. Radiation from electrons in a synchrotron. Phys. Rev. 71:829-830.

Evans, S.D., P.R. Goodrich, R.C. Munter, and R.E. Smith. 1977. Effects of soil and liquid beef manure and liquid hog manure on soil characteristics and on growth, yield, and composition of corn. J. Environ. Qual. 6:361-368.

Fang, F., P.L. Brezonik, D.J. Mulla, and L.K. Hatch. 2002. Estimating runoff phosphorus losses from calcareous soils in the Minnesota River Basin. J. Environ. Qual. 31:1918-1929.

Fendorf, S., D.L. Sparks, G.M. Lamble, and M.J. Kelley. 1994. Application of X-ray absorption fine structure spectroscopy to soils. Soil Sci. Soc. Am. J. 58:1583-1595.

Fendorf, S.E., and D.L. Sparks. 1996. X-ray absorption fine structure spectroscopy. p. 377-416. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, ASA, Inc., Madison, WI.

Flaten, D., K. Snelgrove, I. Halket, K. Buckley, G. Penn, W. Akinremi, B. Wiebe, and Ed. Tyrchniewicz. 2003. Acceptable phosphorus concentrations in soils and impact on the risk of phosphorus transfer from manure amended soils to surface waters. A Review of Literature for the Manitoba Livestock Manure Management Initiative. University of Manitoba and AAFC Brandon Research Centre.

Fordham, A.W., and U. Schwertmann. 1977. Composition and reactions of liquid manure (Gülle), with particular reference to phosphate: II. Solid phase components. J. Environ. Qual. 6:136-139.

Frazer, B.H., B. Gilbert, B.R. Sonderegger, and G. De Stasio. 2003. The probing depth of total electron yield in the sub-keV range: TEY-XAS and X-PEEM. Surf. Sci. 537:161-167.

Freeman, J.S. and D.L. Rowell. 1981. The adsorption and precipitation of phosphate onto calcite. J. Soil Sci. 32:75-84.

Frossard, E., L.M. Condron., A. Oberson., S. Sinaj, and J.C. Fardeau. 2000. Processes governing phosphorus availability in temperate soils. J. Environ. Qual. 29:15-23.

Frossard, E., P. Tekely, and J.Y. Grimal. 1994. Characterization of phosphate species in urban sewage sludge by high-resolution solid-state P-31 NMR. Eur. J. Soil Sci. 45:403-408.

Gale, P.M., M.D. Mullen, C. Cieslik, D.D. Tyle, B.N. Dcuk, M. Kirchner, and J. McClure. 2000. Phosphorus distribution and availability in response to dairy manure applications. Commun. Soil Sci. Plant Anal. 31:553-565.

Gasparatos, D., and C. Haidouti. 2001. A comparison of wet oxidation methods for determination of total phosphorus in soils. J. Plant Nutr. Soil Sci. 164:435-439.

Gburek, W.J., A.N. Sharpley, A.L. Heathwaite, and G.J. Folmar. 2000. Phosphorus management at the watershed scale: a modification of the phosphorus index. J. Environ. Qual. 29:130-144.

Gerritse, R.G., and R. Vriesema. 1984. Phosphorus distribution in animal waste slurries. J. Agric. Sci. 102:159-161.

Ghosh, G.K., K.S. Mohan, and A.K. Sarkar. 1996. Characterization of soil-fertilizer P reaction products and their evaluation as sources of P for gram (*cicer arietinum* L). Nutr. Cycling Agroecosyst. 46:71-79.

Griffin, R.A., and J.J. Jurinak. 1974. Kinetics of the phosphate interaction with calcite. Soil Sci. Soc. Am. Proc. 38:75-79.

Grinsted, M.J., M.J. Hedley, R.E. White, and P.H. Nye. 1982. Plant induced changes in the rhizosphere of rape seedings I. pH change and the increase in P concentration in the soil solution. New Phyto. 91:19-29.

Grossl, P., and W. Inskeep. 1992. Kinetics of octacalcium phosphate crystal growth in the presence of organic acids. Geoch. Cosmochim. Acta. 56:1955-1961.

Grossl, P.R., and W.P. Inskeep. 1991. Precipitation of dicalcium phosphate dihydrate in the presence of organic acids. Soil Sci. Soc. Am. J. 55:670-675.

Güngör, K., A. Jürgensen, and K.G. Karthikeyan. 2007. Determination of phosphorus speciation in dairy manure using XRD and XANES spectroscopy. J. Environ. Qual. 36:1856-1863.

Guo, F., R.S. Yost, N.V. Hue, C.I. Evensen, and J.A. Silva. 2000. Changes in phosphorus fractions in soils under intensive plant growth. Soil Sci. Soc. Am. J. 64:1681-1689.

Halbfass, S., and K. Grunewald. 2003. Spatial variability of phosphorus content in topsoil of two small catchments under agricultural use. J. Plant Nutr. Soil Sci. 166:197-203.

Hansen, J.C., and D.G. Strawn. 2003. Kinetics of phosphorus release from manure-amended alkaline soil. Soil Sci. 168 :869-879.

Hansen, N.C., T.C. Daniel., A.N. Sharpley, and J.L. Lemunyon. 2002. The fate and transport of phosphorus in agricultural systems. J. Soil Water Conserv. 57:408-417.

Hanson, R.L., and D.G. Westfall. 1985. Orthophosphate solubility and availability from dual applied nitrogen and phosphorus. Soil Sci. Soc. Am. J. 49:1283-1289.

Hao, X., F. Godlinski, and C. Chang. 2008. Distribution of phosphorus forms in soil following long-term continuous and discontinuous cattle manure applications. Soil Sci. Soc. Am. J. 72:90-97.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 2005. Soil fertility and fertilizers. 7th ed. Pearson Education. Upper Saddle River, N.J.

He, Z., T.S. Griffin, and C.W. Honeycutt. 2004. Evaluation of soil phosphorus transformations by sequential fractionation and phosphatase hydrolysis. Soil Sci. 169:515-527.

He, Z.L., A.K. Alva, T.C. Li, D.V. Calvert, and D.J. Banks. 1999. Sorption-desorption and solution concentration of phosphorus in a fertilized sandy soil. J. Environ. Qual. 28:1804-1810.

Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46:970-976.

Hedley, M.J., K.J.D. Kirk, and M.B. Santos.1994. Phosphorus efficiency and the forms of soil phosphorus utilized by upland rice cultivars. Plant Soil. 158:53-62.

Heilmann, E., P. Leinweber, G. Ollesch, and R. Meißner. 2005: Spatial variability of sequentially extracted P fractions in a silty loam. J. Plant Nutr. Soil Sci. 168:307-315.

Henriquez, C., and R. Killorn. 2005. Soil P forms and P uptake under intensive plant growth in the greenhouse. Agronomia Costarricense. 29:83-97.

Hesketh, N., and P.C. Brookes. 2000. Development of an indicator for risk of phosphorus leaching. J. Environ. Qual. 29:105-110.

Heslep, J.M., and C.A. Black. 1954. Diffusion of fertilizer phosphorus in soil. Soil Sci. 78:389-401.

Hesterberg, D., W. Zhou, J.K. Hutchinson, S. Beauchemin, and D.E. Sayers. 1999. XAFS study of absorbed and mineral forms of phosphate. J. Synchrotron Rad. 6:636-638.

Hinedi, Z.R., A.C. Chang, and J.P. Yesinowski. 1989. Phosphorus-31 magic angle spinning nuclear magnetic resonance of wastewater sludges and sludges-amended soil. Soil Sci. Soc. Am. J. 53:1053-1056.

Holford, I.C.R., and G.E.G. Mattingly. 1975. The high- and low-energy phosphate adsorbing surfaces in calcareous soils. J. Soil Sci. 26:405-417.

Howard, D.D., E.E. Michael, and L. Joanne. 2002. Long-term broadcast and banded phosphorus fertilization of corn produced using two tillage systems. J. Agron. 95:51-56.

Huang, X.L., and M. Shenker. 2004. Water-soluble and solid-state speciation of phosphorus in stabilized sewage sludge. J. Environ. Qual. 33:1895-1903.

Hunger, S., H. Cho, J.T. Sims, and D.L. Sparks. 2004. Direct speciation of phosphorus in alum-amended poultry litter: Solid-state ^{31}P NMR Investigation. Environ. Sci. Technol. 38:674-681.

Inskeep, W.P., and J.C. Silvertooth. 1988. Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic, and tannic acids. Soil Sci. Soc Am. J. 941-946.

Ippolito, J.A., K.A. Barbarick, D.M. Heil, J.P. Chandler, and E.F. Redente. 2003. Phosphorus retention mechanisms of a water treatment residual. J. Environ. Qual. 32:1857-1864.

Iyamuremye, F., R.P. Dick, and J. Baham. 1996. Organic amendments and phosphorus dynamics: II. Distribution of soil phosphorus fractions. Soil Sci. 161:436-443.

Jackson, B.P., P.M. Bertsch, M.L. Cabrera, J.J. Camberato, J.C. Seaman, and C.W. Wood. 2003. Trace elements speciation in poultry litter. J. Environ. Qual. 32:535-540.

Jackson, M.L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Jiang, C.H., D. Wu, J.W. Hu, F. Liu, X.F. Huang, C.X. Li, and M. Jin. 2010. Application of chemical fractionation and X-ray powder diffraction to study phosphorus speciation in sediments from Lake Hongfeng, China. Chinese Sci. Bull. 56:2098-2108.

Kamprath, E.J., and M.E. Watson. 1980. Conventional soil and tissue tests for assessing the phosphorus status of soils. p. 433-469. *In* F.H. Khasawneh et al. (ed.). The role of phosphorus in agriculture. ASA, CSSA, and SSSA, Madison, WI, U.S.A.

Kar, G., L.S. Hundal, J.J. Schoenau, and D. Peak. 2011. Direct chemical speciation of P in sequential chemical extraction residues using P K-edge XANES spectroscopy. Soil Sci. 176:589-595.

Kashem, M.A., O.O. Akinremi, and G.J. Racz. 2004. Phosphorus fractions in soil amended with organic and inorganic phosphorus sources. Can. J. Soil Sci. 84:83-90.

Katsikini, M., E.C. Paloura, M. Fieber-Erdmann, J. Kalomiros, T.D. Moustakas, H. Amano, and I. Akasaki. 1997. N K-edge X-ray-absorption study of heteroepitaxial GaN films. Phys. Rev. 56:13380-13386.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen-inorganic forms. In: Page, A.L, R.H. Miller, and D.R. Keeney (eds) Methods of soil analysis, 2nd edn. ASA, Madison, pp. 643-698.

Khare, N., D. Hesterberg, and J.D. Martin. 2005. XANES investigation of phosphate sorption in single and binary systems of iron and aluminum oxide minerals. Environ. Sci. Technol. 39:2152-2160.

Khasawneh, F.E., E.C. Sample, and I. Hashimato. 1974. Reactions of ammonium ortho and polyphosphate fertilizer in soil. I. Mobility of phosphorus. Soil Sci. Soc. Am. Proc. 38:446-451.

Khatiwada, R. 2011. Speciation of phosphorus in reduced tillage systems: Placement and source effect. MS thesis. Kansas State University. Manhattan, Kansas, USA.

Kizewski F., Y.T. Liu, A. Morris, and D. Hesterberg. 2010. Spectroscopic approaches for phosphorus speciation in soils and other environmental systems. J. Environ. Qual. 40:751-766.

Kleinman, P.J.A., A.M. Wolf, A.N. Sharpley, D.B. Beegle, and L.S. Saporito. 2005. Survey of water-extractable phosphorus in livestock manures. Soil Sci. Soc. Am. J. 69:701-708.

Kleinman, P.J.A., A.N. Sharpley, B.G. Moyer, and G.F. Elwinger. 2002. Effect of mineral and manure phosphorus sources on runoff phosphorus. J. Environ. Qual. 31:2026-2033.

Kleinman, P.J.A.**,** B.A. Needelman, A.N. Sharpley, and R.W. McDowell. 2003. Using soil profile data to assess phosphorus leaching potential in manured soils. Soil Sci. Soc. Am. J. 67:215-224.

Koenig, R., D. Winward, J. Barnhill, M. Pace, K. Heaton, and C. Reid. 2009. Phosphorus source and surface fluid band spacing effects on irrigated alfalfa. Soil Sci. Soc. Am. J. 73:367-374.

Kruse, J., and P. Leinweber. 2008. Phosphorus in sequentially extracted fen peat soils: A K-edge X-ray absorption near-edge structure (XANES) spectroscopy study. Environ. Sci. Technol. 39:7485-7491.

Kruse, J., W. Negassa, N. Appathurai, L. Zuin, and P. Leinweber. 2010. Phosphorus speciation in sequentially extracted agro-industrial by-products: Evidence from X-ray absorption near edge structure spectroscopy. J. Environ. Qual. 39:2179-2184.

Kumar, V., R.J. Gilkes, and M.D.A. Bolland. 1993. Forms of phosphate in soils fertilized with rock phosphate and super phosphate as measured by chemical fractionation. Aust. J. Soil Res. 31:465-480.

Kumaragamage, D., O.O. Akinremi, D. Flaten, and J. Heard. 2007. Agronomic and environmental soil test phosphorus in manured and non-manured Manitoba soils. Can. J. Soil Sci. 87:73-83.

Laboski, C.A.M., and J.A. Lamb. 2003. Changes in soil test phosphorus concentration after application of manure or fertilizer. Soil Sci. Soc. Am. J. 67:544-554.

Larsen, S. 1967. Soil phosphorus. Adv. Agron. 19:151-210.

Lawton, K., and J.A. Vomocil. 1954. The Dissolution and migration of phosphorus from granular superphosphate in some Michigan soils. Soil Sci. Soc. Am. Proc. 18:26-32.

LECO© Corporation. 1987. Instruction manual for CR-12 carbon system. St. Joseph, MI. 59p.

Lehmann, J., Z. Lan, C. Hyland, S. Sato, D. Solomon, and Q. Ketterings. 2005. Long-term dynamics of phosphorus forms and retention in manure-amended soils. Environ. Sci. Technol. 39:6672-6680.

Leinweber, P. 1996. Phosphorus fractions in soils from an area with high density of livestock population. Z. P. Flanzenern. Bodenk. 159:251-256.

Leinweber, P., B.L. Turner, and R. Meissner. 2002. The role of phosphorus in agriculture and environmental pollution. pp. 29-55. *In* Haygarth, P.M., and S.C. Jarvis (eds) Agriculture, hydrology and water quality. CABI Publ., Wallingford, UK.

Lightsources.org. 2007. [Online] Available at: www.lightsources.org/cms/?pid=1000098

Lindsay, W.L. 1979. Chemical equilibria in soils. Wiley-Interscience, New York.

Lombi, E., K.G. Scheckel, R.D. Armstrong, S. Forrester, J.N. Cutler, and D. Paterson. 2006. Speciation and distribution of phosphorus in a fertilized soil: A synchrotron-based investigation. Soil Sci. Soc. Am. J. 70:2038-2048.

Magid, J., H. Tiessen, and L.M. Condron. 1996. Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. *In* "Humic substances in terrestrial ecosystems" (A. Piccolo, Ed.), pp. 429-466. Elsevier Science, Oxford.

Maguire, R.O., J.T. Sims, and F.J. Coale. 2000. Phosphorus fractionation in biosolids-amended soils: Relationship to soluble and desorbable phosphorus. Soil Sci. Soc. Am. J. 64:2018-2024.

Maguire, R.O., J.T. Sims, W.W. Saylor, B.L. Turner, R. Angel, and T.J. Applegate. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual. 33:2306-2316.

Mattingly, G.E.G. 1975. Labile phosphate in soils. Soil Sci. 119:369-375.

McDonald, A.J. 2000. The effects of cover crops on soil N transformations and losses from arable land. Ph.D. thesis, The University of Reading, UK.

McDowell, R.W., and A.N. Sharpley. 2001. Phosphorus losses in subsurface flow before and after manure application to intensively farmland. Sci. Total Environ. 278:113-125.

McKenzie, R.H., J.W.B. Stewart, J.F. Dormaar, and G.B. Schaalje. 1992. Long-term crop rotation and fertilizer effects on phosphorus transformations: II. In a Luvisolic soil. Can. J. Soil Sci. 72:581-589.

Meek, B.D., L.E. Graham, T.J. Donovan, and K.S. Mayberry. 1979. Phosphorus availability in calcareous soil after high loading rates of animal manure. Soil Sci. Soc. Am. J. 43:741-743.

Menon, R.G., S.H. Chein, and G. Abd el Nabi. 1991. Comparison of Olsen and P_i soil tests for evaluating phosphorus bioavailability in a calcareous soil treated with single superphosphate and partially acidulated phosphate rock. Fert. Res. 29:153-158.

MnKeni, P.N.S., and A.F. MacKenzie. 1985. Retention of ortho and polyphosphates in some Quebec soils as affected by added organic residues and calcium carbonate. Can. J. Soil Sci. 65:575-585.

Moody, P.W., D.G. Edwards, and L.C. Bell. 1995. Effect of banded fertilizers on soil solution composition and short-term root growth: II. Mono and di-ammonium phosphates. Aust. J. Soil Res. 33:689-707.

Mooleki, P., J.J. Schoenau, G. Hultgreen, G. Wen, and J.L. Charles. 2002. Effect of rate, frequency and method of liquid swine manure application on soil nitrogen availability, crop performance and nitrogen use efficiency in east-central Saskatchewan. Can. J. Soil Sci. 82:457-467.

Moorby H., R.E. White, and P.H. Nye. 1988. The influence of phosphate nutrition on H ion efflux from the roots of young rape plants. Plant Soil. 105:247-256.

Moore, P.A., Jr., T.C. Daniel, A.N. Sharpley, and C.W. Wood. 1995. Poultry manure management: Environmentally sound options. J. Soil Water Conserv. 50:321-327.

Morel, C., H. Tunney, D. Plenet, and S. Pellerin. 2000. Transfer of phosphate ions between soil and solution. Perspective in soil testing. J. Environ. Qual. 29:50-59.

Mozaffari M., and J.T. Sims. 1996. Phosphorus transformations in poultry litter amended soils of the Atlantic coastal plain. J. Environ. Qual. 25:1357-1365.

Muir, J.W. 1952. The determination of total phosphorus in soil. Analyst. 77:313-317.

Mulkey, J.R., E.L. Albach, and F.J. Daniel. 1979. Response of onions to P placement. Agron. J. 71:1037-1040.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for determination of phosphorus in natural waters. Anal. Chim. Acta. 27:31-36.

Nair, V.D., D.A. Graetz, and K.M. Portier. 1995. Forms of phosphorus in soil profiles from dairies of south Florida. Soil Sci. Soc. Am. J. 59:1244-1249.

Negassa, W., and P. Leinweber. 2009. How does the Hedley sequential P fractionation reflect impacts of land use and management on soil phosphorus: A review. J. Plant Nutr. Soil Sci. 172:305-325.

Negassa, W., J. Kruse, D. Michalik, N. Appathurai, L. Zuin, and P. Leinweber. 2010. Phosphorus speciation in agro-industrial byproducts: Sequential fractionation, solution P-31 NMR, and P K- and L-2, L-3-edge XANES spectroscopy. Environ. Sci. Technol. 44:2092-2097.

Nelson, D.W., and T.J. Logan. 1983. Chemical processes and transport of phosphorus. *In* F.W. Schaller and G.W. Bailey (eds) Proc. National Conf. on Agric. Manage, and Water Qual. Iowa State University Press, Ames, IA.

Newville, M. 2003. Fundamentals of XAFS. Consortium of advanced radiation Sources. University of Chicago. Chicago, IL.

O'Halloran, I.P. 1993. Effect of tillage and fertilization on inorganic and organic soil phosphorus. Can. J. Soil Sci. 73:359-369.

O'Halloran, I.P., J.W.B. Stewart, and R.G. Kachanoski. 1987. Influence of texture and management practices on the forms and distribution of soil phosphorus. Can. J. Soil Sci. 67:147-163.

Ohno, T., and B.S. Crannell. 1996. Green and animal manure-derived dissolved organic matter effects on phosphorus sorption. J. Environ. Qual. 25:1137-1143.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. pp. 403-430. *In*: A.L. Page et al.(ed), Methods of Soil Analysis. 2nd ed. Agron Ser. 9, Part-2. SSSA, Madison, WI.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture, Washington, DC. Circular 939.

Parham, J.A., S.P. Deng, W.R. Raun, and G.V. Johnson. 2002. Long-term cattle manure application in soil I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biol. Fertil. Soils. 35:328-337.

Peak, D., G. Kar, L. Hundal, and J. Schoenau. 2012. Kinetics and mechanisms of phosphorus release in a soil amended with biosolids or inorganic fertilizer. Soil Sci. 177:183-187.

Peak, D., J.T. Sims, and D.L. Sparks. 2002. Solid-state speciation of natural and alum-amended poultry litter using XANES spectroscopy. Environ. Sci. Technol. 36:4253-4261.

Penn, C.J., and R.B. Bryant. 2008. Phosphorus solubility in response to acidification of dairy manure amended soils. Soil Sci. Soc. Am. J. 72:238-243.

Peters, J., S. Combs, B. Hoskins, J. Jarman, J. Kovar, M. Watson, A. Wolf, and N. Wolf. 2003. Recommended methods for manure analysis. Madison, WI, USA.

Pierson, S.T., M.L. Cabrera, G.K. Evanylo, H.A. Kuykendall, C.S. Hoveland, M.A. McCann, and L.T. West. 2001. Phosphorus and ammonium concentrations in surface runoff from grasslands fertilized with broiler litter. J. Environ. Qual. 30:1784-1789.

Pierzynski, G.M., J.T. Sims, and G.F. Vance. 2000. Soils and environmental quality. 2nd ed. CRC Press, Boca Raton, FL.

Pote, D.H., T.C. Daniel, A.N. Sharpley, P.A. Moore, D.E. Edwards, and D.J. Nicholos. 1996. Relating extractable soil phosphorus to phosphorus losses in runoff. Soil Sci. Soc. Am. J. 60:855-859.

Pote, D.H., T.C. Daniel, D.J. Nichols, A.N. Sharpley, P.A. Moore, Jr., D.M. Miller, and D.R. Edwards. 1999. Relationship between phosphorus runoff levels in three Ultisols and phosphorus concentrations in runoff. J. Environ. Qual. 28:170-175.

Prummel, J. 1957. Fertilizer placement experiments. Plant Soil. 8:231-253.

Qian, P, J.J. Schoenau, and W.Z. Huang. 1992. Use of ion exchange membrane in routine soil testing. Commun. Soil Sci. Plant Anal. 23:179-1804.

Qian, P., and J.J. Schoenau. 2000a. Fractionation of P in soil as influenced by a single addition of liquid swine manure. Can. J. Soil Sci. 80:561-566.

Qian, P., and J.J. Schoenau. 2000b. Use of ion exchange membrane to assess soil N supply to canola as affected by addition of liquid swine manure and urea. Can. J. Soil Sci. 80:213-218.

Qian, P., and J.J. Schoenau. 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. Can. J. Soil Sci. 82:9-21.

Qian, P., J.J. Schoenau, and R.E. Karamanos. 1994. Simultaneous extraction of available phosphorus and potassium with a new soil test: A modification of Kelowna extraction. Commum. Soil Sci. Plant Anal. 25:627-635.

Qian, P., J.J. Schoenau, T. Wu, and P. Mooleki. 2004. Phosphorus amounts and distribution in a Saskatchewan soil after five years of swine and cattle manure application. Can. J. Soil Sci. 84:275-281.

Richards, J.E., T.E. Bates, and S.C. Sheppard. 1995. Changes in the forms and distribution of soil phosphorus due to long term corn production. Can. J. Soil Sci. 75:311-318.

Rose, T.J., B. Hardiputra, and Z. Rengel. 2010. Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant Soil. 326:159-170.

Ryan, J., D. Curtin, and M.A. Cheema. 1984. Significance of iron oxide and calcium carbonate particle size in phosphate sorption by calcareous soils. Soil Sci. Soc. Am. J. 48:74-76.

Safley Jr, L. M., P.W. Westerman, J.C. Barker, L.D. King, and D.T. Bowman. 1986. Slurry dairy manure as a corn nutrient source. Agric. Wastes. 18:123-136.

Sample, E.C., R.J. Soper, and G.J. Racz. 1980. Reactions of phosphate fertilizers in soils. Pages 263-310 *in* F.E. Khasawneh., E.C. Sample, and E.J. Kamprath, eds. The role of phosphorus in agriculture. ASA-CSSA-SSSA, Madison, WI.

Sanchez, C.A., P.S. Porter, and M.F. Ulloa. 1991. Relative efficiency of broadcast and banded phosphorus for sweet corn produced on Histosols. Soil Sci. Soc. Am. J. 55:871-875.

Sander, D.H., E.J. Penas, and B. Eghball. 1990. Residual effects of various phosphorus application methods on winter wheat and grain sorghum. Soil Sci. Soc. Am. J. 54:1473-1478.

SAS Institute. 2011. SAS/STAT users' guide. Version 9.2. SAS Inst., Cary, NC

Saskatchewan Agriculture and Food. 2003. Tri-Provincial manure application and use guidelines. Government of Saskatchewan, Regina, SK, Canada.

Saskatchewan Ministry of Agriculture. 2006. Available at: http://www.agriculture.gov.sk.ca/Default.aspx?DN=3a8c4fbe-e1ed-48f3-ae85-9340b45d1a39

Saskatchewan Soil Survey. 1989. Rural Municipality of Humboldt No. 370. Preliminary soil map and report. Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, SK, Canada.

Sato, S., D. Solomon, C. Hyland, Q.M. Ketterings, and J. Lehmann. 2005. Phosphorus speciation in manure and manure-amended soils using XANES spectroscopy. Environ. Sci. Technol. 39:7485-7491.

Sawyer, J.E., M.A. Schmitt, and R.D. Hoeft. 1990. Inorganic nitrogen distribution and soil chemical transformations associated with injected liquid beef manure. Agron. J. 82:963-969.

Schlichting, A., P. Leinweber, R. Meissner, and M. Altermann. 2002. Sequentially extracted phosphorus fractions in peat-derived soils. J. Plant Nutr. Soil Sci. 165:290-298.

Schoenau, J.J, J. Charles, R.P. Wen, P. Qian, and G. Hultgreen. 1999. Hog and cattle manure additions in the black soil zone of Saskatchewan: agronomic considerations. Pages 59-70 *in* Proceeding 1999 Soil and Crops Workshop, University of Saskatchewan, Saskatoon, SK.

Schoenau, J.J., and W.Z. Huang. 1991. Anion-exchange membrane, water, and sodium bicarbonate extractions as soil tests for phosphorus. Commum. Soil Sci. Plant Anal. 22:465-492.

Schoenau, J.J., J.W.B. Stewart, and J.R. Bettany. 1989. Forms and cycling of phosphorus in prairie and boreal forest soils. Biogeochem. 8:223-237.

Schoenau. J.J., and J.R. Bettany. 1987. Organic matter leaching as a component of carbon, nitrogen, phosphorus, and sulfur cycles in a forest, grassland, and gleyed soil. Soil Sci. Soc. Am. J. 51:646-651**.**

Seiter, J.M., K.E. Staats-Borda, M. Ginder-Vogel, and D.L. Sparks. 2008. XANES spectroscopic analysis of phosphorus speciation in alum-amended poultry litter. J Environ. Qual. 37:477-485.

Shadi, A., H. Tiessen, and J. Scheoenau. 2010. Correlations between phosphorus fractions and total leachate phosphorus from cattle manure- and swine manure-amended soil. Commun. Soil Sci. Plant Anal. 41:1338-1349.

Sham, T.K. and M. Rivers, 2002. A brief overview of synchrotron radiation. p. 117-147. *In* P. Fenter., M. Rivers., N. Sturchio, and S. Sutton (eds) Applications of synchrotron radiation in low-temperature geochemistry and environmental science, Vol. 49 of Reviews in Mineralogy and Geochemistry. The Mineralogical Society of America, Washington, DC.

Shand, C.A., G. Coutts, and S. Hillier. 2005. Phosphorus composition of sheep feces and changes in the field determined by P-31 NMR spectroscopy and XRPD. Environ. Sci. Technol. 39:9205-9210.

Sharpley, A., T.C. Daniel, J.T. Sims, and D.H. Pote. 1996. Determining environmentally sound soil phosphorus levels. J. Soil Water Conserv. 51:160-166.

Sharpley, A.N. 1985. Depth of surface soil-runoff interaction as affected by rainfall, soil slope, and management. Soil Sci. Soc. Am. J. 49:1010-1015.

Sharpley, A.N., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. J. Environ. Qual. 29:1462-1469.

Sharpley, A.N., S.C. Chapra, R. Wedepohl, J.T. Sims, T.C. Daniel, and K.R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: Issues and options. J. Environ. Qual. 23:437-451.

Sharpley, A.N., S.J. Smith, B.A. Steward, and A.C. Mathers. 1984. Forms of phosphorus in soil receiving cattle feedlot manure waste. J. Environ. Qual. 13:211-215.

Sharpley, A.N., S.J. Smith, O.R. Jones, W.A. Berg, and G.A. Coleman. 1992. The transport of bioavailable phosphorus in agricultural runoff. J. Environ. Qual. 21:30-35.

Sharpley, A.N., U. Singh, G. Uehara, and J. Kimble. 1989. Modeling soil and plant phosphorus dynamics in calcareous and highly weathered soils. Soil Sci. Soc. Am. J. 53:153-158.

Shober, L.A., D.L. Hesterberg, J.T. Sims, and S. Gardner. 2006. Characterization of phosphorus species in biosolids and manures: Using XANES spectroscopy. J. Environ. Qual. 35:1983-1993.

Sibbesen, E., and A.N. Sharpley. 1997. Setting and justifying upper critical limits for phosphorus in soils. p. 151-176. *In* H. Tunney et al. (ed.) Phosphorus loss from soil to water. CAB Int., London.

Simard, R.R., D. Cluis, G. Gangbazo, and S. Beauchemin. 1995. Phosphorus status of forest and agricultural soils from a watershed of high density. J. Environ. Qual. 24:1010-1017.

Simard, R.R., S. Beauchemin, I. Royer, and G.M. Barnett. 2001. Manure impact on phosphorus transfer from soils to surface waters. p. 21-33. *In* Proc. Alberta Soil Sci. Workshop, 38th, Lethbridge, AB, Canada. 20-22 Feb. 2001. Alberta Agric. Food and Rural Development, Edmonton.

Sims, J.T., A.C. Edwards, O.F. Schoumans, and R.R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural management practices. J. Environ. Qual. 29:60-71.

Sims, J.T., R.R. Simard, and B.C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27:277-293.

Singh, D.K., P.W.G. Sale, and R.R. Routley. 2005. Increasing phosphorus supply in subsurface soil in northern Australia: Rationale for deep placement and the effects with various crops. Plant Soil. 269:35-44.

Sleight, D.M., D.H. Sanders, and G.A. Peterson. 1984. Effect of fertilizer phosphorus placement on the availability of phosphorus. Soil Sci. Soc. Am. J. 48:336-340.

Smillie, G.W., and J.K. Syers. 1972. Fluoride formation during extraction of calcareous soils with fluoride II. Implications to Bray-1 test. Soil Sci. Soc. Am. Proc. 36:25-30.

Soil Classification Working Group. 1998. The Canadian System of Soil Classification. 3rd ed. NRC Research Press.

Sommers, L.E., and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil Sci. Soc. Am. Proc. 36:902-904.

Stumborg, C.M. 2006. Phosphorus and nitrogen loading in manured Saskatchewan soils. MS thesis. University of Saskatchewan, Saskatoon, SK, Canada.

Sui, Y., M.L. Thompson, and C. Shang. 1999. Fractionation of phosphorus in a Mollisol amended with biosolids. Soil Sci. Soc. Am. J. 63:1174-1180.

Sumner, M.E., and W.P. Miller. 1996. Cation exchange capacity and exchange coefficients. Pages 1201-1229 *in* D.L. Sparks, ed. Methods of soil analysis. Part-3. Chemical methods. ASA, SSSA, Madison, WI.

Sutton, A.L., D.W. Nelson, J.D. Hoff, and V.B. Mayrose. 1982. Effects of injection and surface application of liquid swine manure on corn yield and soil composition. J. Environ. Qual. 11:468-472.

Syers, J.K., J.D.H. Williams, and T.W. Walker. 1968. The determination of total phosphorus in soils and parent materials. N.Z. J. Agric. Res. 11:757-762.

Tamenori, Y. 2011. Soft X-ray photoabsorption spectroscopy using fluorescence yield method. Beamline Practice at BL27SU, Japan Synchrotron Radiation Research Institute. Hyogo, Japan.

Thomas, R.L., R.W. Sheard, and J.R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agron. J. 59:240-248.

Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction. pp. 75-86 *in* M.R. Carter, ed. Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, FL.

Tiessen, H., J. W. B. Stewart, and C.V. Cole. 1984. Pathways of phosphorus transformations in soils of differing pedogenesis. Soil Sci. Soc. Am. J. 48:853-858.

Toor, G.S., D.J. Peak, and J.T. Sims. 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. J. Environ. Qual. 34:687-697.

Toor, G.S., S. Hunger, J.D. Peak, J.T. Sims, and D.L. Sparks. 2006. Advances in the characterization of phosphorus in organic wastes: Environment and agronomic applications. Advan. Agron. 89:1-72.

Torbert, H.A., T.C. Daniel, J.L. Lemunyon, and R.M. Jones. 2002. Relationship of soil test phosphorus and sampling depth to runoff phosphorus in calcareous and noncalcareous soils. J. Environ. Qual. 31:1380-1387.

Traina, S.J., G. Sposito, D. Hesterberg, and U. Kafkafi. 1986. Effects of pH and organic acids on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Sci. Soc. Am. J. 50: 45-52.

Tran, T.S., and A. N'dayegamiye. 1995. Long-term effects of fertilizers and manure application on the forms and availability of soil phosphorus. Can. J. Soil Sci. 75:28I-285.

Tunesi, S., V. Poggi, and C. Gessa. 1999. Phosphate adsorption and precipitation in calcareous soils: The role of calcium ions in solution and carbonate minerals. Nutr. Cycling Agroecosyst. 53:219-227.

Tunney, H., and B. Pommel. 1987. Phosphorus uptake by ryegrass from monocalcium phosphate and pig manure on two soils in pots. Ir. J. Agric. Res. 26:189-198.

Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 33:757-766.

Turner, B.L., and A.B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manures: Chemical speciation and a novel fractionation procedure. Environ. Sci. Technol. 38:6101-6108.

Vadas, P.A., W.J. Gburek, A.N. Sharpley, P.J.A. Kleinman, P.A. Moore, M.L. Cabrera, and R.D. Harmel. 2007. A model for phosphorus transformation and runoff loss for surface-applied manures. J. Environ. Qual. 36:324-332.

Van der Paauw, F. 1971. An effective water extraction method for the determination of plant-available soil phosphorus. Plant Soil. 34:467-481.

Van Lierop, W. 1988. Determination of available phosphorus in acid and calcareous soils with the Kelowna multi-element extract. Soil Sci. 146:284-291.

Vogel, G., and D.L. Sparks. 2010. The impacts of X-ray absorption spectroscopy on understanding soil chemical processes and reaction mechanisms, p 1-26. *In* B. Singh and M. Grafe (eds). Development in soil science 34: Synchrotron-based technique in soils and sediments. Elsevier, Burlington, MA.

Wagar, B.I., J.W.B. Stewart, and J.O. Moir. 1986. Changes with time in the form and availability of residual fertilizer phosphorus on chernozemic soils. Can. J. Soil Sci. 66:105-119.

Wakene, N., and P. Leinweber. 2009. How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. J. Plant Nutr. Soil Sci. 172:305-325.

Whalen, J.K., and C. Chang. 2001. Phosphorus accumulation in cultivated soils from long-term annual applications of cattle feedlot manure. J. Environ. Qual. 30:229-239.

Winick, H. 1994. Synchrotron radiation sources: a primer. World Scientific, River Edge, NJ.

Wright, C.R., M. Amrani, A.T. Jedrych, A. Atia, D. Heaney, and D.S. Vanderwel. 2003. Phosphorus loading of soil through manure application and subsequent transport with runoff: The P-mobility study. Alberta Agriculture, Food, and Rural Development. Edmonton, AB.

www.lightsource.ca/education/resource.php

Zamuner, E.C., L.I. Picone, and H.E. Echeverria. 2008. Organic and inorganic phosphorus in Mollisol soil under different tillage practices. Soil and Tillage Res. 99:131-138.

Zhang, H., G. Johnson, and M. Fram. 2002. Managing phosphorus from animal manure. Division of Agricultural Services and Natural Resources. Oklahoma Cooperative and Extension Services. Okalahoma, USA.

Zheng, Z., J.A. Macleod, J.B. Sanderson, and J. Lafond. 2004. Soil phosphorus dynamics after ten annual applications of mineral fertilizers and liquid dairy manure: fractionation and path analyses. Soil Sci. 169:449-456.

Zheng, Z., R.R. Simard, J. Lafond, and L.E. Parent. 2001. Changes in phosphorus fractions of a humaic gleysol as influenced by cropping systems and nutrient sources. Can. J. Soil Sci. 81:175-183.

APPENDIX A

Table A.1. Distribution of the different P forms at 1, 3, and 5 weeks in the presence of plants after addition of solid cattle manure (SCM) and liquid hog manure (LHM) amended soil.

Factor		Resin-P	$NaHCO_3$-P_i	$NaHCO_3$-P_o	NaOH-P_i	NaOH-P_o	HCl-P	Residual-P
Treatment		mg P kg^{-1} soil						
Control		52.83b§	25.52b	29.78b	49.58b	190.4b	147.9c	90.04b
SCM		284.8a	99.85a	56.80a	79.26a	274.5a	249.5a	111.0a
LHM		66.69b	28.50b	42.10b	51.95b	261.0a	219.4b	88.52b
Time (week)								
1st week		108.6b	32.22b	39.83b	44.92b	259.2b	227.1a	98.90a
3rd week		129.6b	55.17a	32.84b	48.65b	288.6a	184.5c	88.49a
5th week		166.1a	66.18a	56.01a	87.22a	178.1c	205.2b	102.1a
*Treatment*Time*								
Control	1st week	29.21cd	19.35c	28.93b	37.65cd	188.0c	207.37c	96.38a
	3rd week	49.69cd	27.66bc	17.42b	40.56cd	262.1b	123.47d	89.38a
	5th week	79.59bc	29.55bc	43.00b	70.51b	120.9d	112.87d	84.38a
SCM	1st week	271.0a	54.53b	42.74b	52.80c	298.7ab	250.1ab	112.4a
	3rd week	289.3a	111.8a	45.40b	69.59b	320.6a	220.0bc	97.73a
	5th week	294.0a	132.3a	82.26a	115.4a	204.2c	278.4a	122.7a
LHM	1st week	25.47d	22.77c	47.81b	44.29cd	290.7ab	223.8bc	87.93a
	3rd week	49.82cd	26.07c	35.71b	35.79d	283.1ab	210.1c	78.37a
	5th week	124.8b	36.66bc	42.77b	75.76b	209.1c	224.4bc	99.27a

§Means followed by different letters in column are significantly different at $P \leq 0.05$ for each factor.

APPENDIX B

Table B.1. Annual and cumulative amounts of total N applied in 11 years of either solid cattle manure (SCM) or liquid hog manure (LHM) application at low and high rate.

		Annual total N applied											Total N input
Manure	Treatment	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
		kg P ha^{-1}											
SCM	Low	121	104	69	113	76	177	81	81	73	73	73	1041
	High	484	416	276	452	304	708	324	324	292	292	292	4164
LHM	Low	74	51	97	94	87	118	40	84	49	68	68	830
	High	295	204	390	376	348	472	160	336	196	272	272	3320